Taibah University

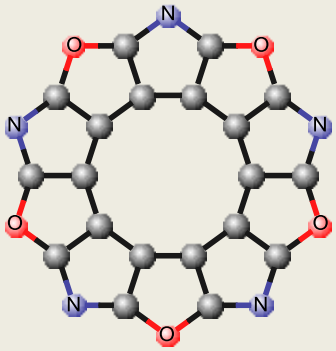
College of Pharmacy

Practical

PHARMACEUTICAL CHEMISTRY III

By

Dr. Hany Emary Ali Ahmed



Al-Madinah Al-Munawarah

1443 – 2021

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Syllabus

1. **Molecular modeling**
2. **Molecular representations(2D and 3D)**
   - **2.1.** Connectivity table
   - **2.2.** SMILE string
   - **2.3.** 3D representations
3. **Energy minimization**
   - **3.1.** Potential energy calculation
4. **Flexible alignment**
5. **Molecular properties calculation**
   - **5.1.** Log P value
   - **5.2.** Lipinski's rule
6. **Ligand-target interaction**
   - **6.1.** Types of interactions
   - **6.2.** Cases study
7. **Pharmacophore analysis**
   - **7.1.** Definition and types
   - **7.2.** Cases study
8. **QSAR**
   - **8.1.** Definition
   - **8.2.** Cases study
9. **Molecular docking**
   - **9.1.** Definition
   - **9.2.** Cases study

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Molecular Modeling

Drug design is an iterative process which begins when a chemist identifies a compound that displays an interesting biological profile and ends when both the activity profile and the chemical synthesis of the new chemical entity are optimized.

Drug design has two major classes:

1. **Indirect drug design "ligand-based"**:

This relies on knowledge of other molecules that bind to the biological target of interest. These other molecules may be used to derive 'a pharmacophore' model that defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target. In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be based on the knowledge of what binds to it, and this model in turn may be used to design new molecular entities that interact with the target.

2. Direct drug design "Structure-based":

Which relies on knowledge of the three dimensional structure of the biological target obtained through methods such as x-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available it may be possible experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist or various automated computational procedures to suggest new drug candidates.

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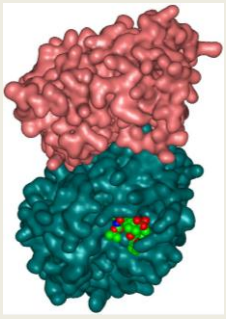
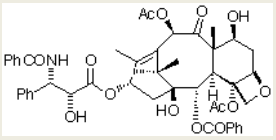
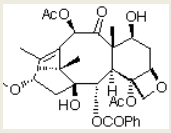
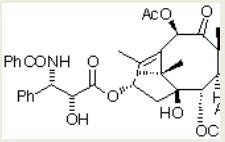
Drug Design Approaches







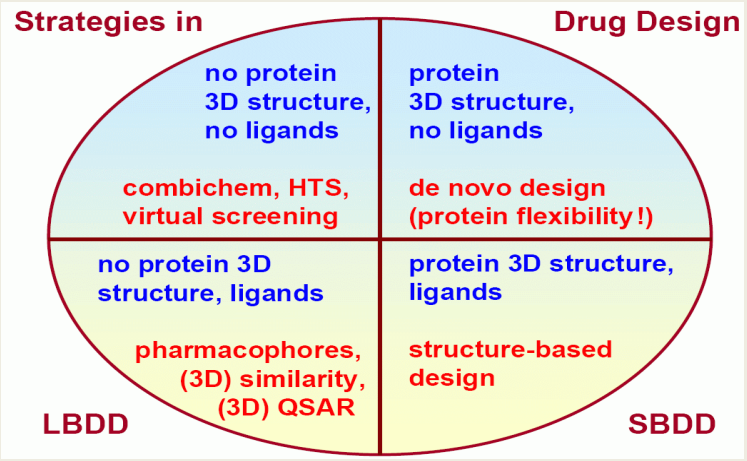


Biological Targets

- Docking
- Pharmacophore

Ligands

- Pharmacophore analysis
- QSAR analysis



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Molecular representations

The starting point for many computer assisted molecular design (CAMD) studies is generally a two dimensional drawing of a compound of interest. These diagrams can range from notebook or "back-of-the-envelope" sketches to electronically stored connection tables in which one defines the types of atoms in the molecule, their hybridization and how they are bonded to each other. Carbon dioxide, for example, would be defined as one SP2 oxygen atom (atom number 1) bonded to an SP carbon atom (atom number 2) with a double bond which in turn, is bonded to a second SP2 oxygen atom with a double bond. The connection table which defines carbon dioxide might be written as shown down.

atom #	Atom Name	Atom Type	Bound to Atoms
1	O	5	2
2	C	2	1, 3
3	O	5	2

Figure 1. Connection Table for Carbon Dioxide

Connection tables are easily stored and searched electronically. However, they must be transformed into three dimensional representations of chemical structure to study chemical properties. Chemists use the mathematical descriptions of the rules of physical chemistry which are contained in quantum mechanics and molecular mechanics to accomplish this task.

Aim: we introduce some of the methods used for the computational representation of molecular structures and the creation of structural databases.

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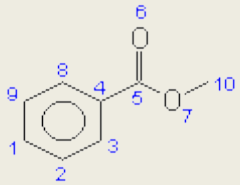
A. 2D structure representations

1. The connection Table

Name of constant	Value	Description
btAromatic	4	aromatic bond
btDouble	2	double bond
btNoBond	0	bond undefined
btSingle	1	single bond
btTriple	3	triple bond

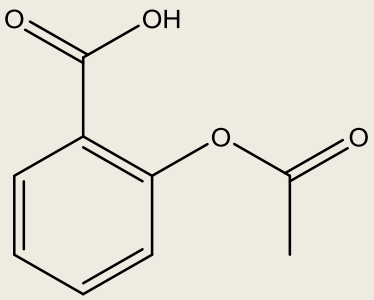


index	Atomic number	Atom type	Connected to atom	bond type
1	6	C	2, 9	4, 4
2	6	C	1, 3	4, 4
3	6	C	2, 4	4, 4
4	6	C	3, 5, 8	4, 1, 4
5	6	C	4, 6, 7	1, 2, 1
6	8	O	5	2
7	8	O	5, 10	1, 1
8	6	C	4, 9	4, 4
9	6	C	1, 8	4, 4
10	6	C	7	1

Figure2. The connection table for aspirin in the MDL format (hydrogen-suppressed form). The numbering of the atoms is as shown in the chemical diagram.

- **Build the table for the following compounds :**

Aspirin



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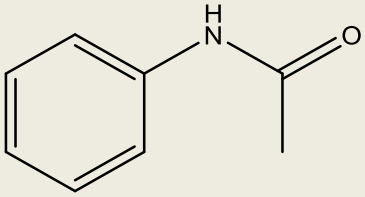
Acetanilide

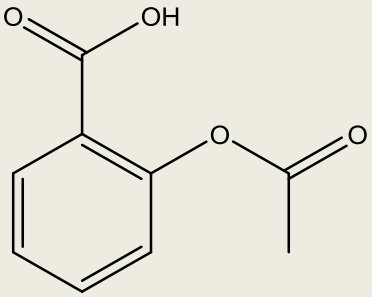


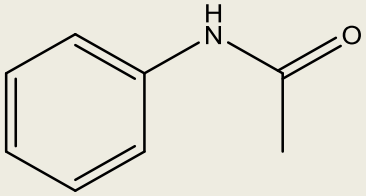
2. SMILES STRING

A SMILES string is composed of atom specifications arranged sequentially according to connectivity in the molecular graph.

Example

O(C(=O)C)c1ccccc1C(O)=O

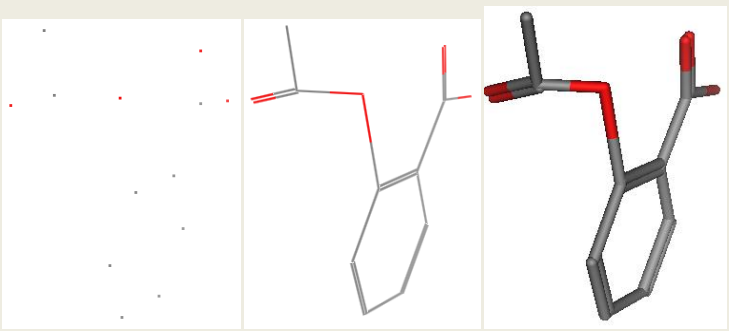




- **Draw structure ---→ Edit ----→ Copy as (2D MDL, SMILES)**

B. 3D structure representations

- **Types of modes : Point/ Line /stick / Ball and line / Ball and stick/Space filling**



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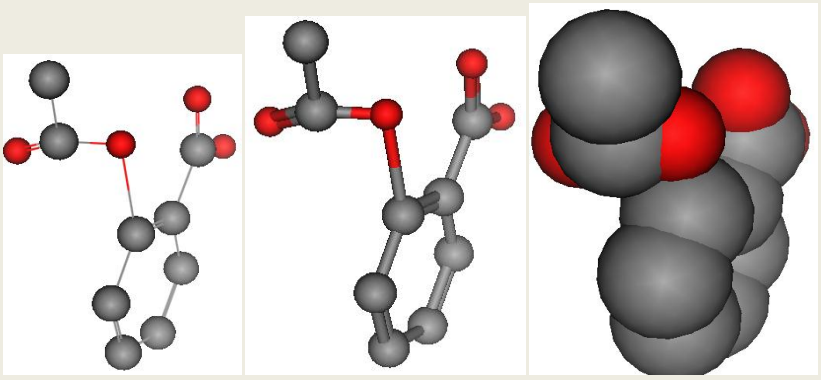

- **Draw structure ---→ Mode -→ what ---→ Edit → Copy as (3D MDL, export images as BMP)**
- **Record the xyz coordinates of each atom and try to change it**

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Energy minimization

Aim: The Energy Minimize application in MOE calculates atomic coordinates that are local minima of a molecular energy function. Energy minimization is useful for molecule building, determining low energy conformations, conformational search and preparation for molecular dynamics simulations and vibrational analysis

In summary: Energy minimization can be used to find a reasonable and energetically preferred 3D conformation of the molecule.

Steps:

a. Summarize in your own words how energy minimization basically works.

b. Open the SVL window to monitor the following computation steps. Measure the initial potential energy of your ligand.

$$E(x) = E_{str} + E_{ang} + E_{stb} + E_{oop} + E_{tor} + E_{vdw} + E_{ele} + E_{sol} + E_{res}$$

- E_{str} is the bond stretch energy
- E_{ang} is the bond angle bend energy
- E_{stb} is the stretch-bend energy
- E_{oop} is the out-of-plane energy
- E_{tor} is the dihedral angle, or torsion energy
- E_{vdw} is the van der Waals energy
- E_{ele} is the electrostatics energy
- E_{sol} is the solvation energy
- E_{res} is the restraint energy

c. Perform an energy minimization on your ligand. How does the conformation of the molecule change? **What is the potential energy of the minimized structure?** Which terms show the most significant changes? Can you give reasons for that? Save the minimized structure as moe file.

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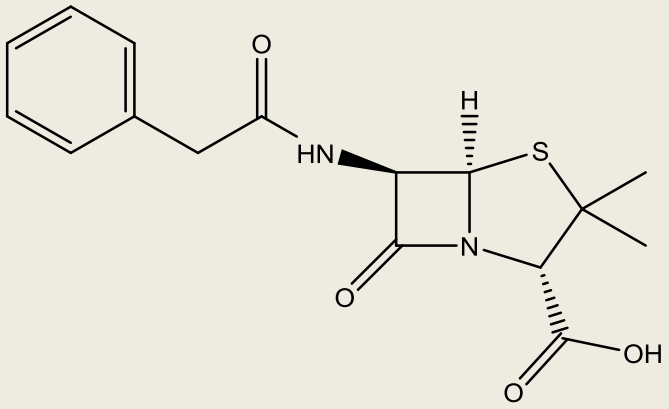
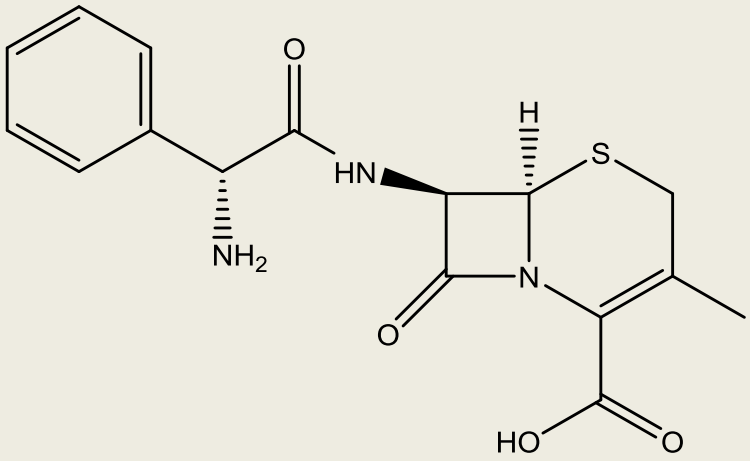
How to apply:

- **Compute >>> potential energy -----record the value of the total energy of the ligand**
- **Compute >>> energy minimize**
- **Compute >>> potential energy ----- record the value of the NEW total energy of the ligand**

Penicillin G



Cephalexin



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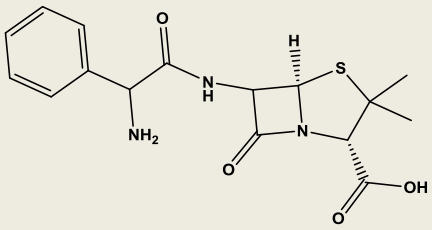
Identification and assay of penicillins

Ampicillin



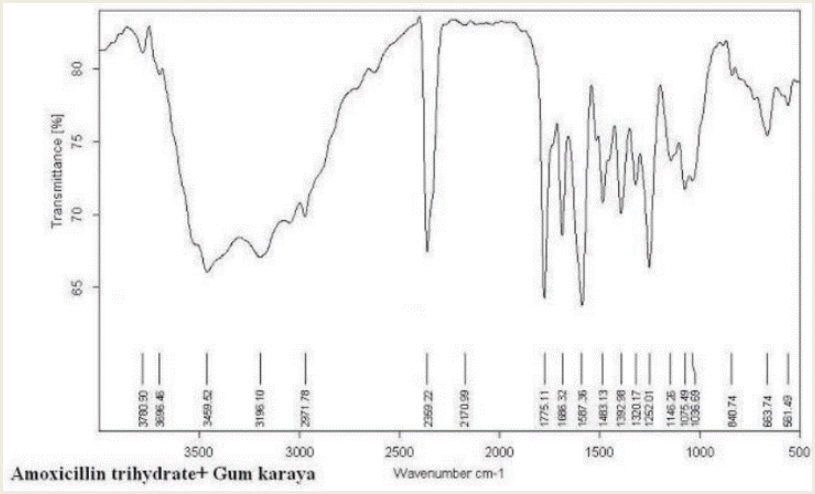
1. **Functional group analysis: -**

COOH , NH_2 , C=O

a. Reaction with Na_2CO_3 ---------- eff. with evolving of CO_2.
b. Reaction with $FeCl_3$ ---------- Colored solution or precipitate.

2. **Spectroscopic identification of the drug**

IR



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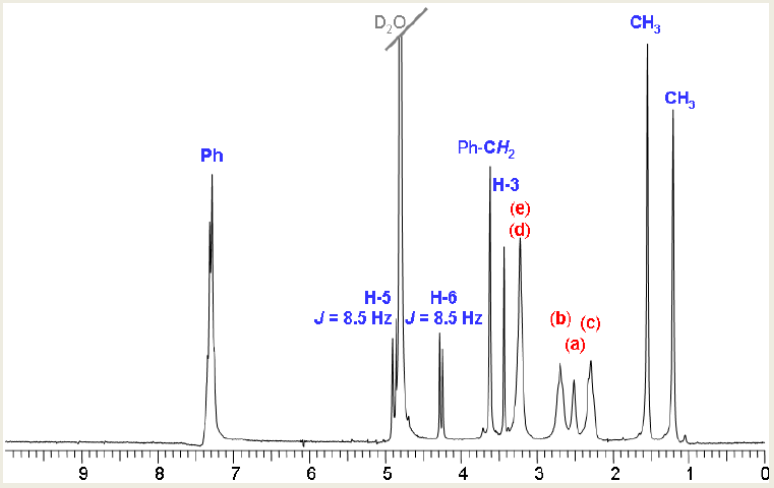
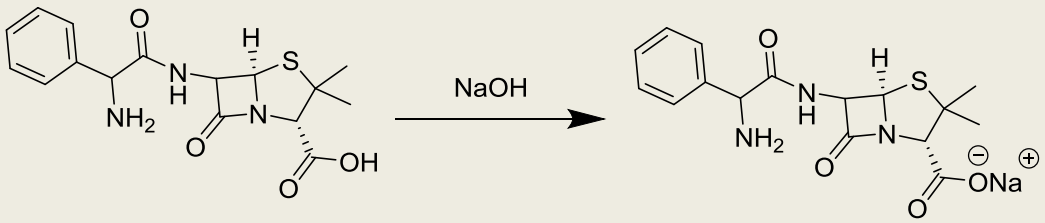
HNMR



3. Assay of the drug: using acid-base titrations

Ampicillin solution in acetonitrile-water mixture is titrated with standard sodium hydroxide solution. The end point is determined by pH measurement or using of Phenolphthalein as indicator.



Principle: Ampicillin is semi synthetic penicillin, which is used as a broad-spectrum antibiotic. The principle involved in assay is acidimetric titration. Ampicillin is stable to acids & being acidic in nature it can be assayed directly by titrating with base. It is a monobasic acid, which consists of one carboxylic acid, and therefore it consumes 1mole of NaOH solution. Thus, it can be estimated by no. of moles of NaOH consumed in the titration. The titration is done with NaOH after dissolving the sample in the free water dissolving the sample in free water (freshly boiled and cooled).

Procedure:

- Weigh accurately quantity of powder equivalent to 0.5g of Ampicillin.

- Transfer the powder into a conical flask containing 25ml of freshly boiled and cooled distilled water.

- This solution aside for 10min and then add phenolphthalein indicator. Titrate the solution with 0.1 N NaOH until permanent pink color is obtained.

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• Equivalent Factor: 1 ml of 0.1 N NaOH = **0.03495 gm** of Ampicillin.

• Report: The amount of ampicillin present in the given sample was found to be ----------.

• % purity was found to be----------.

Test yourself:

If the mls of titrant = 14 ml

Ampicillin weight in gm =

% purity =

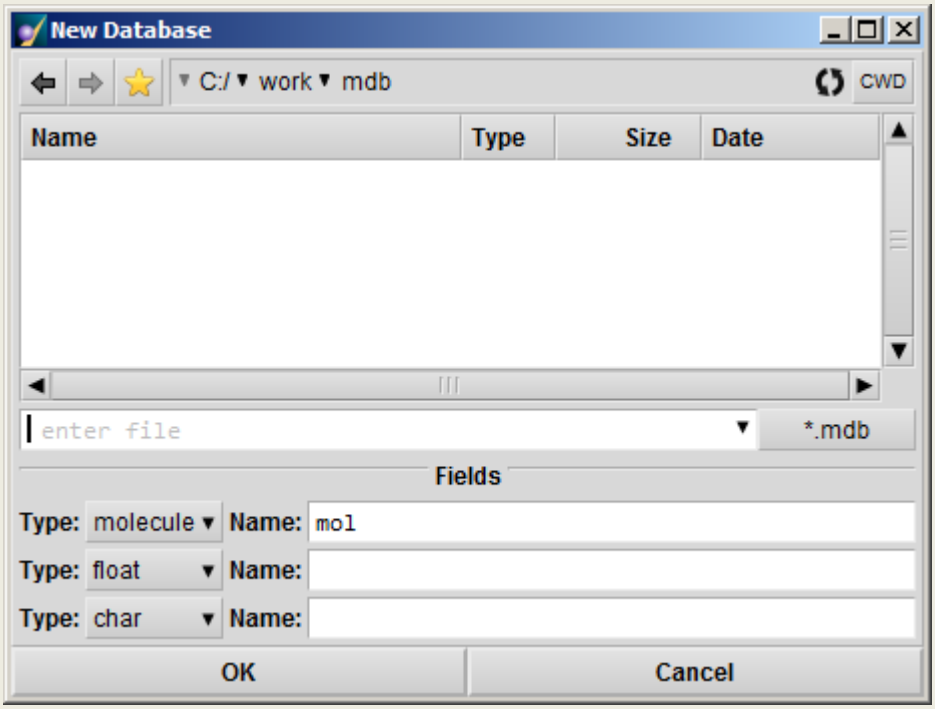
Database creation

This chapter explains the basic steps involved in creating and editing databases containing numeric, text and molecular data entries. If you are looking for information on a specific menu or command, click on the links in the Database Viewer Menu Bar.

The MOE interface provides several ways of creating databases:

- In the MOE Window (and also in the Sequence Editor), choose File | New | Database.
- In the Database Viewer, choose DBV | File | New.

The following panel appears:



In the **New Database** panel, specify the path and name of the new database. The lower portion of the panel allows you to create three fields in the new database. If the name fields are left blank then no fields will be created. By default, a single molecule field is created with the field name of **mol**.

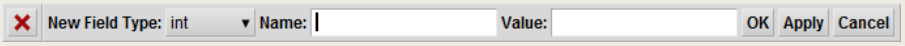
Press **OK** to create the new database and open it in a Database Viewer.

Creating Fields

Fields hold numeric, character, or molecular data. Operations on fields are done via the Edit menu or the Field Popup menu (position the cursor over the field name and press the mouse popup button).

1. In the Database Viewer, choose DBV | Edit | New | Field. You can also use the Field Popup menu: DBV | Field Popup | New.

   The CLI prompt area will display an option menu with the available database data types and a text field for entering the new field name. Non-molecule fields (i.e. numeric or character) can also take a value. The value will be replicated in the new field across all entries.



2. Select the type of field to create:

`int`	Numeric	Integer value or vector of integers
`float`	Numeric	Real number or vector of real numbers (4-byte IEEE floating point data)
`char`	Text	String of characters
`byte`	Numeric	8-bit numerical value ranging from `-128` to `127` inclusive
`molecule`	Molecular	MOE molecular format (see mol_Extract)
`double`	Numeric	Real number or vector of real numbers (8-byte IEEE floating point data)

3. Enter the name of the new field and, if desired, a value used to initialize the field. Press `Return` to create the field. The field names appear as column labels in the top row of the database. You can also use the **Apply** button to create the field. Using the **OK** button will create the field and dismiss the prompter.
4. Repeat this procedure to create as many fields as required by the database you are creating. Once this is done, you can add entries to the database, as described below.

The Field Popup | Duplicate command can also be used to create a new field. In this case, an exact copy of the field is created. You will be asked to confirm the field creation. The name of the new field is automatically generated from the original field name by prefixing it with `'Copy of '`. If a field of this name already exists, the new field will be numbered (e.g. `'Copy (2) of...'`). The new field is positioned immediately to the right of the original field.

Multiple fields can be duplicated using the Field Popup | Selection | Duplicate command. The copied fields are appended, i.e. are positioned as the rightmost columns of the database.

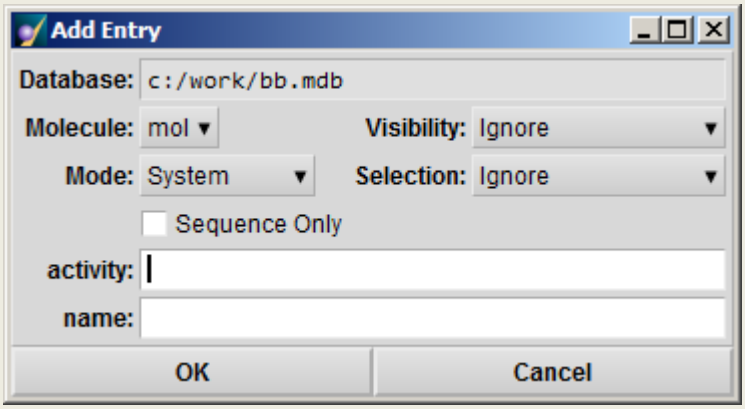
Adding Entries

Entries are commonly added to a database automatically through many of MOE's applications. However, it is often required to add entries manually to a database in order to store data currently viewed in the MOE Window. This section describes how to add entries to a database and how to filter the system in order to copy only parts of the system into the new database cells.

To open the Add Entry panel use either of the following commands:

- DBV | Edit | New | Entry
- DBV | Entry Popup | New

A panel similar to the following appears:



The panel consists of two sections: the **Molecular Field** and the **Non-Molecular Fields**. If there are no fields of the specified type then that section will not be displayed.

To add a single new entry with no data initialization, set the **Mode** option to **None** and press **OK**. To add a single new entry with the entire system copied into the specified molecule field, simply press **OK**.

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QSAR Tutorial

This tutorial has been broken down into the following steps:

I. Calculating Molecular Descriptors
II. Fitting the Experimental Data
III. Cross-Validating the Model
IV. Performing Graphical Analysis
V. Estimating the Predicted Activities for the Test Set
VI. Pruning the Descriptors
VII. References

The Quantitative Structure-Activity Relationship (QSAR) suite of applications in MOE is used to analyze experimental data and build numerical models of the data for prediction and interpretation purposes. Given a set of molecules whose activity in a particular experiment is known (referred to as a training set or learning set), a QSAR model correlates these activities with properties inherent to each molecule in the set. These properties are evaluated using molecular descriptors available in MOE.

The particular example chosen for this exercise is the Selectivity Differences of Inhibitors Binding to Trypsin [Bohm, 1999; Stürzebecher, 1991, 1993, 1995]. The entire training data set consists of 72 molecules derived from 3-amidinophenylalanine and the test set has 16 molecules similarly derived. The molecules in the training set are aromatic and polar, and properties such as molar refractivity and the logarithm of the (octanol/water) partition coefficients (logP) are important in describing such systems.

Molecular descriptors are calculated using QuaSAR-Descriptor.

In this tutorial you will learn how to:

1. Choose appropriate molecular descriptors, evaluate them for each molecule in the training set and store them in a database.
2. Fit the experimental activity to these descriptors using a linear model.
3. Cross-validate the fit and perform graphical analysis.
4. Estimate the activities of a set of related molecules known as the test set.
5. Prune a set of descriptors to obtain a more relevant descriptor set using QuaSAR-Contingency.

Conventions Used in This Tutorial

MOE	MOE Window
SE	Sequence Editor
DBV	Database Viewer

This tutorial assumes that you are using a three-button mouse. For information on using a two-button mouse see Using the Mouse.

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I. Calculating Molecular Descriptors

1. Open the database that contains the training data set with experimental activities:

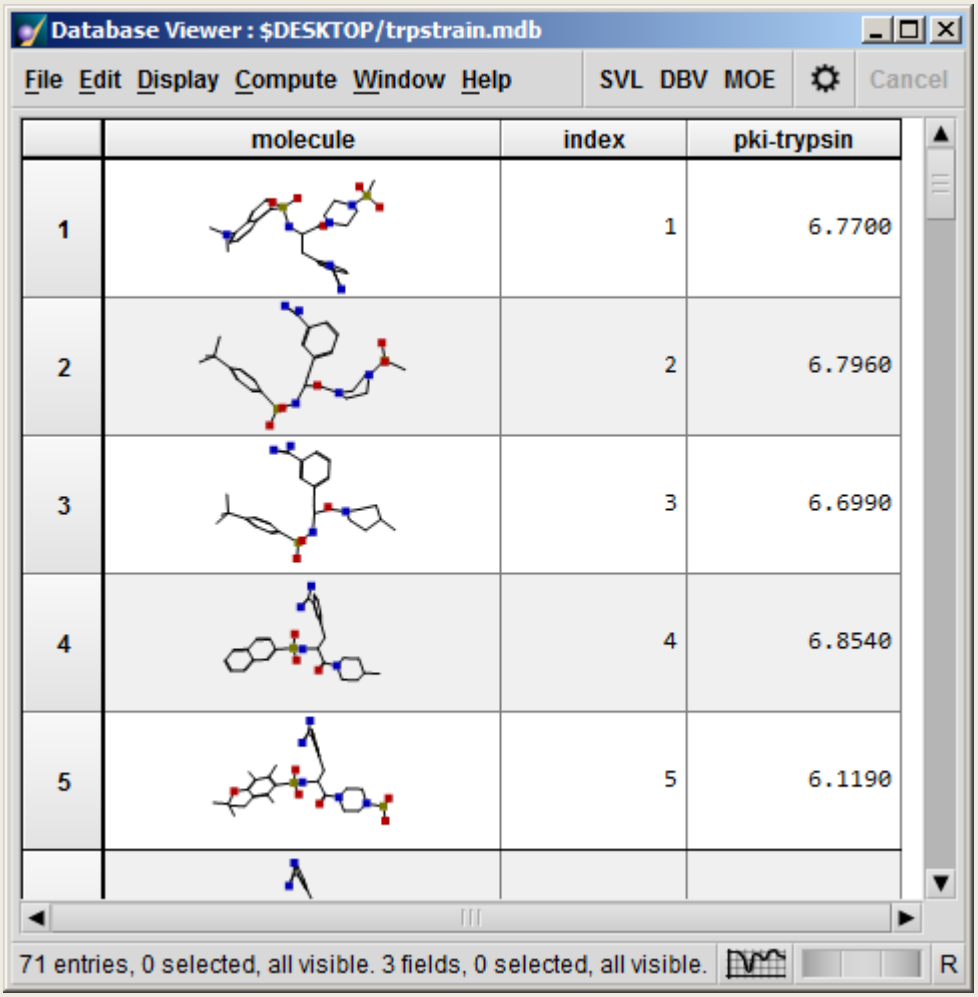
   MOE | File | Open | `$MOE/sample/mol/trpstrain.mdb`

2. Because this database is read-only, you must save it in your own working directory in order to run calculations on the data:

   DBV | File | Save As | `your local directory/mytrpstrain.mdb`

   The training set consists of 71 molecules derived from 3-amidinophenylalanine. It contains the structure and activity (pK_i) of each molecule. The **index** field is the molecule number given in the original paper.

   To get a better view of the molecules in the database, position the cursor over any one of the cells in the first column (called **molecule**), press the left mouse button and drag down (and slightly to the right) until you clearly see all the molecular drawings. You can rotate each drawing by dragging the middle mouse button over it.



3. The next step in a QSAR analysis is to choose suitable molecular descriptors:

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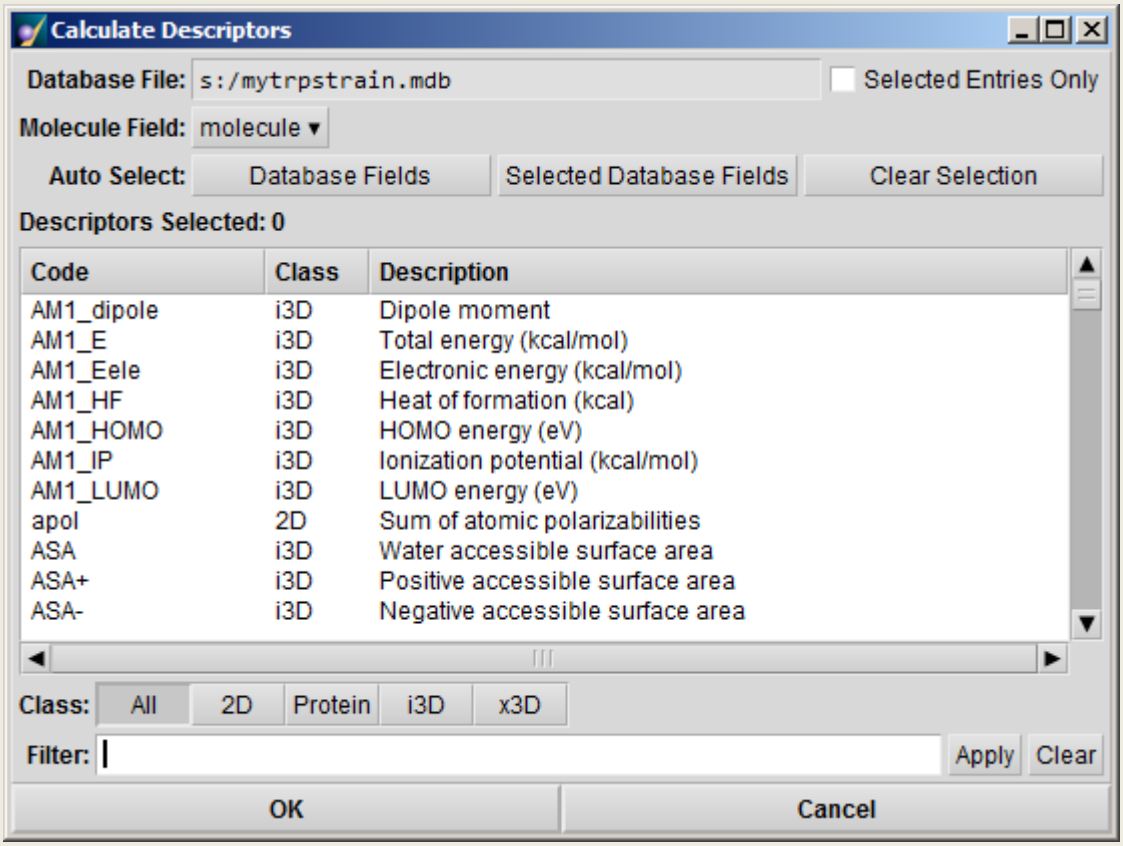
DBV | Compute | Descriptors | Calculate

The Calculate Descriptor panel includes the list of all the descriptors available in MOE:



The following 18 descriptors will be used:

```
SlogP_VSA0                    SMR_VSA0
SlogP_VSA1                    SMR_VSA1
SlogP_VSA2                    SMR_VSA2
SlogP_VSA3                    SMR_VSA3
SlogP_VSA4                    SMR_VSA4
SlogP_VSA5                    SMR_VSA5
SlogP_VSA6                    SMR_VSA6
SlogP_VSA7                    SMR_VSA7
SlogP_VSA8
SlogP_VSA9
```

An easy way to select the descriptors is to use the **Filter** at the bottom of the panel:

a. To select the first column of descriptors, enter `sl` in the Filter text field. Only descriptors with the characters `sl` are listed. Click on `SlogP_VSA0`, press `Shift` and click on `SlogP_VSA9`. Notice that the **Descriptors Selected** count now indicates that 10 descriptors are selected.

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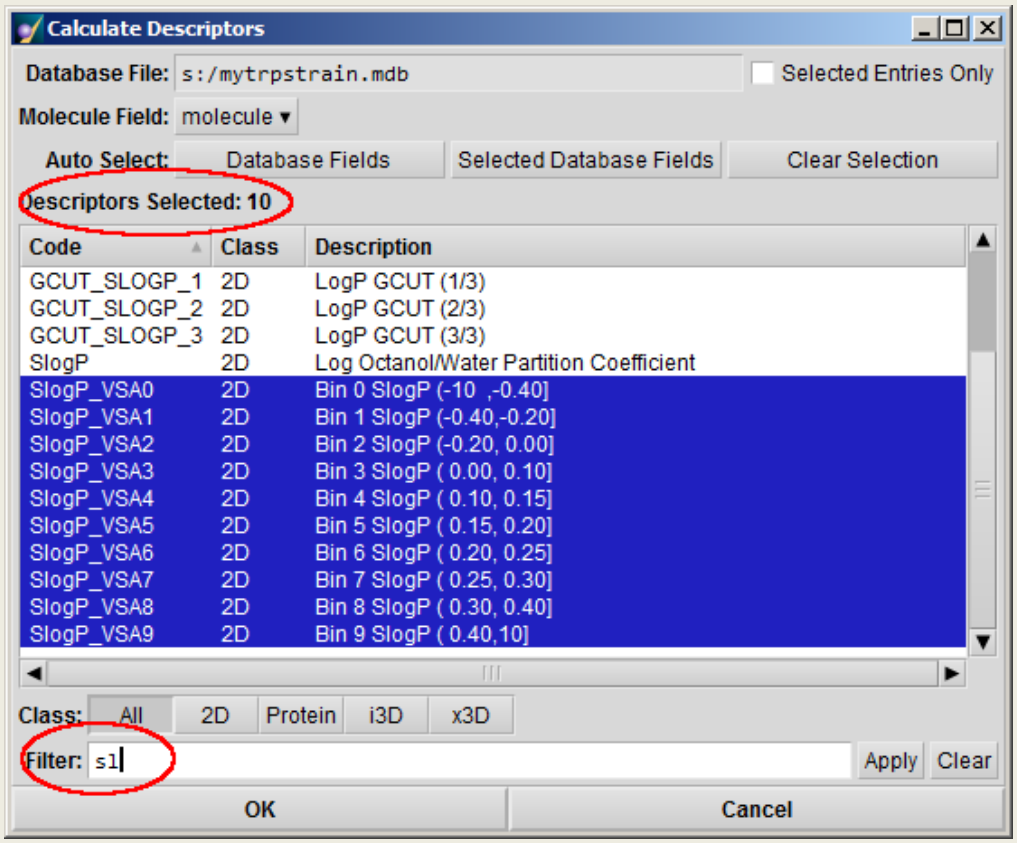

Calculate Descriptors
Database File: s:/mytrpstrain.mdb
Selected Entries Only
Molecule Field: molecule
Auto Select: Database Fields
Selected Database Fields
Clear Selection
Descriptors Selected: 10
Code
Class
Description
GCUT_SLOGP_1 2D LogP GCUT (1/3)
GCUT_SLOGP_2 2D LogP GCUT (2/3)
GCUT_SLOGP_3 2D LogP GCUT (3/3)
SlogP 2D Log Octanol/Water Partition Coefficient
SlogP_VSA0 2D Bin 0 SlogP (-10 ,-0.40]
SlogP_VSA1 2D Bin 1 SlogP (-0.40,-0.20]
SlogP_VSA2 2D Bin 2 SlogP (-0.20, 0.00]
SlogP_VSA3 2D Bin 3 SlogP (0.00, 0.10]
SlogP_VSA4 2D Bin 4 SlogP (0.10, 0.15]
SlogP_VSA5 2D Bin 5 SlogP (0.15, 0.20]
SlogP_VSA6 2D Bin 6 SlogP (0.20, 0.25]
SlogP_VSA7 2D Bin 7 SlogP (0.25, 0.30]
SlogP_VSA8 2D Bin 8 SlogP (0.30, 0.40]
SlogP_VSA9 2D Bin 9 SlogP (0.40,10]
Class: All 2D Protein i3D x3D
Filter: sl
Apply
Clear
OK
Cancel

b. Now enter `sm` in the Filter text field. Only descriptors with these characters appear. Select the series of `SMR_VSA` descriptors using `Shift` (the descriptors selected previously remain selected even though they are not currently visible in the panel). The panel should report that 18 descriptors have been selected.

A brief description appears beside each descriptor. For more information, see QuaSAR-Descriptors.

4. Press **OK** once the set has been selected. MOE will evaluate each descriptor for every molecule and store the values in `mytrpstrain.mdb`. New fields are named based on the descriptor codes.
5. Now suppose you need to add a new molecule (e.g. trypsin72 which has a pK_i of 3.0) to the database and would like to calculate its descriptors as well. Fortunately, it is not necessary to re-select and re-evaluate all the descriptors in the Calculate Descriptors panel as was done previously. Instead, you can tell MOE to evaluate the descriptors for the new database entry only.

a. Open the molecule into MOE:

MOE | File | Open | `$MOE/sample/mol/trypsin72.moe`

b. Add the new molecule to `mytrypstrain.mdb`:

DBV | Edit | New | Entry

The Add Database Entry panel lists all the fields in the database. Next to the molecule field are buttons specifying which atoms to copy from MOE. All other database fields have text fields where you can enter values. To add the molecule to the database:

- Enter its **index** number: `72`.
- Enter its **pki-trypsin** value: `3.0`.
- Press **OK**. The molecule is appended to the database, becoming entry number 72.
- Remove the molecule from the MOE system using MOE | RHS | Close.

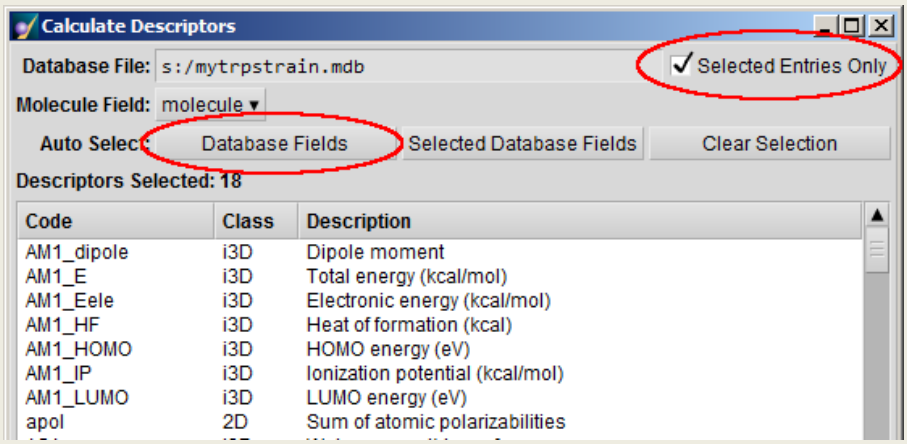
c. Before calculating the descriptors for this molecule, you must first select the new entry in the database. To do so, simply click on the entry number, i.e. number 72 in the left-most column.

d. Reopen the Calculate Descriptor panel:

DBV | Compute | Descriptors | Calculate

In the Calculate Descriptor panel:

- Turn on the **Selected Entries Only** option.
- Click on **Auto Select: Database Fields**. This automatically reselects (in the Calculate Descriptor panel) all of the descriptors that were previously calculated in the database. Notice that the line above the list states: **Descriptors Selected: 18**.



- Ensure that entry 72 is the only selected entry in the database and click **OK** to calculate the values for the descriptors of the new molecule. Then deselect entry 72:

DBV | Edit | Clear | Entry Selection

II. Fitting the Experimental Data

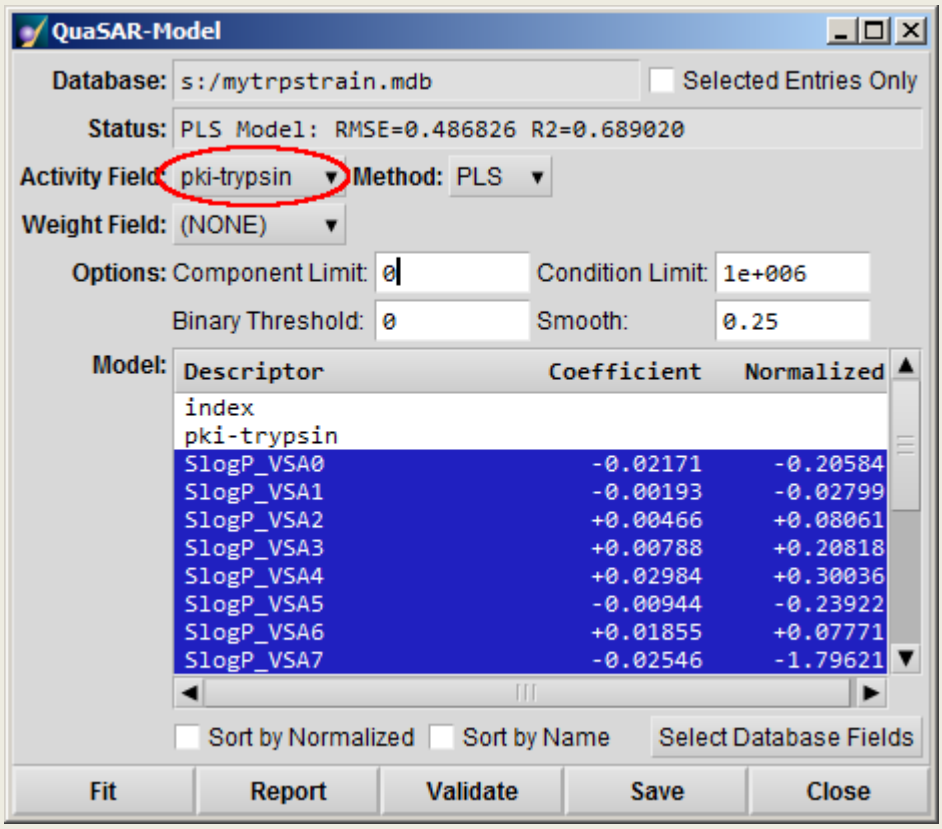
The next step is to fit the dependent variable, `pki-trypsin`, to the independent variables, namely the molecular descriptors, in the database.

1. Open the QuaSAR-Model panel:

   DBV | Compute | Model | QSAR

2. In the panel:

Practical Pharmaceutical Chemistry III



a. As **Activity Field**, select: `pki-trypsin`.
b. Choose all the descriptors except for `pki-trypsin` and `index`.

Tip To select a range of descriptors in the list, click on the first item, `SlogP_VSA0`, scroll to the end of the list and press `Shift` as you click on the last item, `SMR_VSA7`. This selects the entire range of descriptors.

c. Press **Fit** to perform the regression analysis. The root mean square error (RMSE) and r^2 (R2) values of the fit appear at the top of the QuaSAR-Model panel, in the Status field.
d. Press **Report** to view a report on the calculations in the SVL Text Editor window. You can save or discard the text file before closing the SVL Text Editor.
e. Press **Save** in the QuaSAR-Model panel, and save the model as `trypsinpred.fit`. This model will be used to predict the activities of the candidates in the test data set.

Do not close the QuaSAR-Model panel yet.

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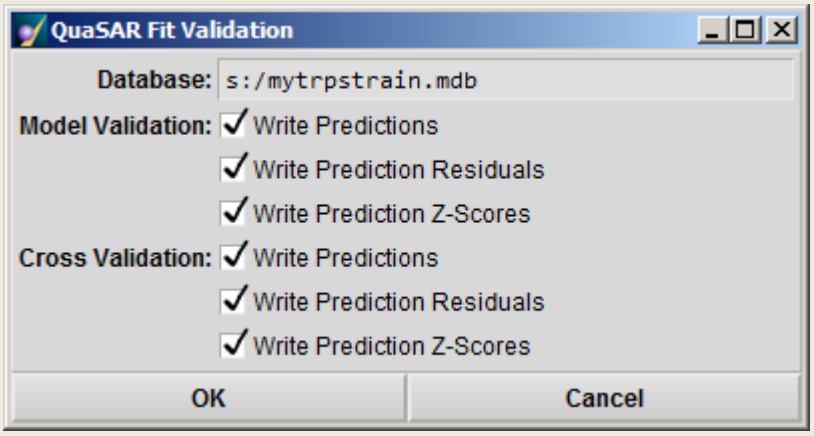
III. Cross-Validating the Model

Having performed the QSAR fit, it is now useful to validate the model. The validation procedure evaluates the predicted activities and the residuals for the molecules in the training set. In cross-validation, the model is tested using a LOO (**L**eave **O**ne **O**ut) method.

1. Click on **Validate** in the QuaSAR-Model panel. The QuaSAR Fit Validation panel appears:



2. Select all the Model Validation and Cross Validation properties and press **OK**. (You can now close the QuaSAR-Model panel.)

$PRED, **$RES** and **$Z-SCORE** fields are created in `mytrpstrain.mdb`. They contain the predicted, residual and Z-score values respectively. The corresponding cross-validation properties, **$XPRED**, **$XRES** and **$XZ-SCORE**, are also calculated. (More information is available in QuaSAR-Model.)

IV. Performing Graphical Analysis

A $Z-SCORE value of 2.5 and above can be considered to indicate molecules which are outliers to the fit. These might require new descriptors. You can graphically display which molecules are outliers in the database using MOE's plotting facilities.

1. To display the plot area in the bottom half of the Database Viewer:

   Toggle on DBV | $Z-SCORE Field Popup | Plot or the **Plot** icon in the database viewer footer.

2. You can plot the $Z-SCORE values using the Plot Fields drop-down menu:

   Plot Fields | $Z-SCORE

3. Click the **Fit** button in the database viewer footer. This adjusts the display so that all data points are visible in the plot area. Each dot represents an entry (in other words, a molecule in the database).

As you can see, two dots exceed the 2.5 mark. (Since there are only two outliers in this example, the choice of descriptors can be considered adequate.) Position the cursor over a dot without moving for a second or so. The number of the entry will appear in yellow bubble help. Select the dots by clicking left in the vertical ruler at 2.5, and dragging upward. This causes a selection box to appear. Releasing the mouse causes all points in the selection box to become selected. (You can also drag a selection box in the plot area itself.)

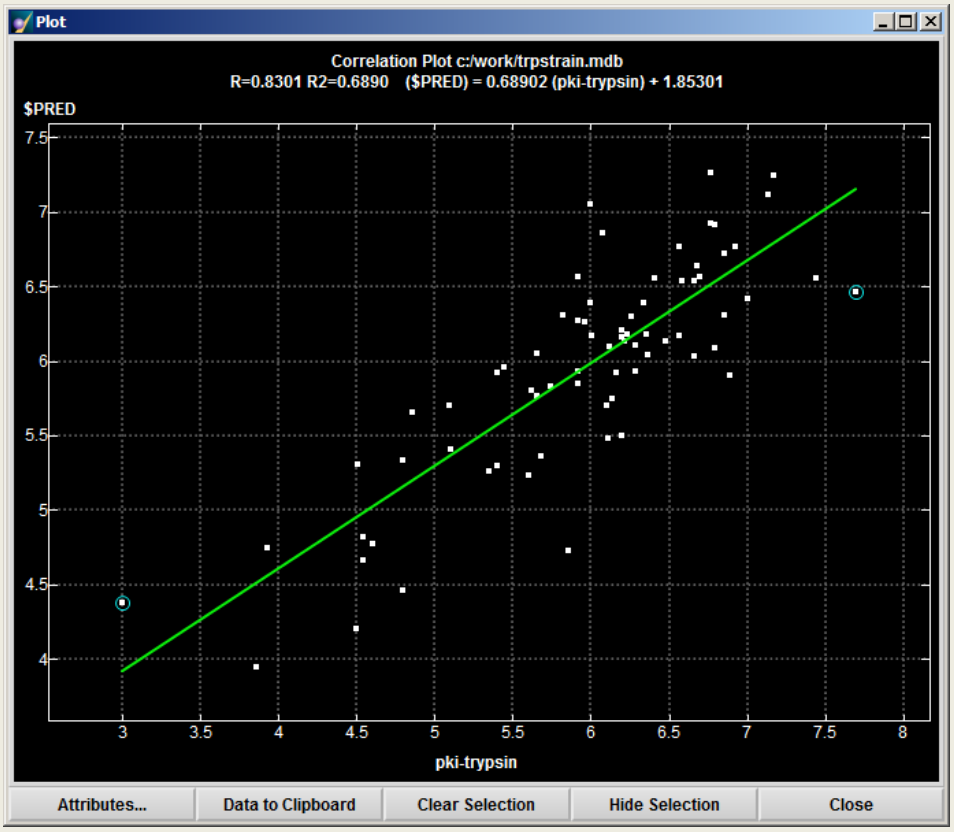
4. Another type of plot used to evaluate the data results is the XY plot. By plotting the actual activities (X-axis) versus the predicted activities (Y-axis), the predictive ability of the model can be assessed. To show the linear relationship between actual and predicted activities, open the correlation plot panel:

   DBV | Compute | Analysis | Correlation Plot

   and select:

   a. Select a field to plot in the X-axis: choose pKi-trypsin
   b. Select a field to plot in the Y-axis: choose $PRED

   The correlation plot will be generated as shown below:



   As shown in the above correlation plot, a linear relationship exists between the actual and predicted activities. The correlation coefficient is displayed in the top of the plot. To further establish the reliability of the QSAR model, apply the model against an external test set to evaluate the predictive ability.

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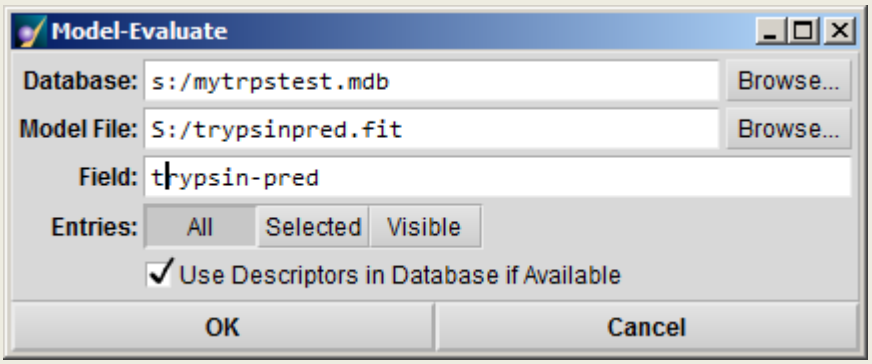
V. Estimating the Predicted Activities for the Test Set

Using the predicted fit `trypsinpred.fit` obtained above, you can now evaluate the predicted pK_i values of the test set of molecules. The test set includes 16 molecules derived from 3-amidinophenylalanine.

1. **DBV | File | Open | `$MOE/sample/mol/trpstest.mdb`**
2. Because this database is read-only, you must save it in your own working directory in order to run calculations on the data:

   DBV | File | Save As | `your local directory/mytrpstest.mdb`

3. **DBV | Compute | Model | Evaluate**

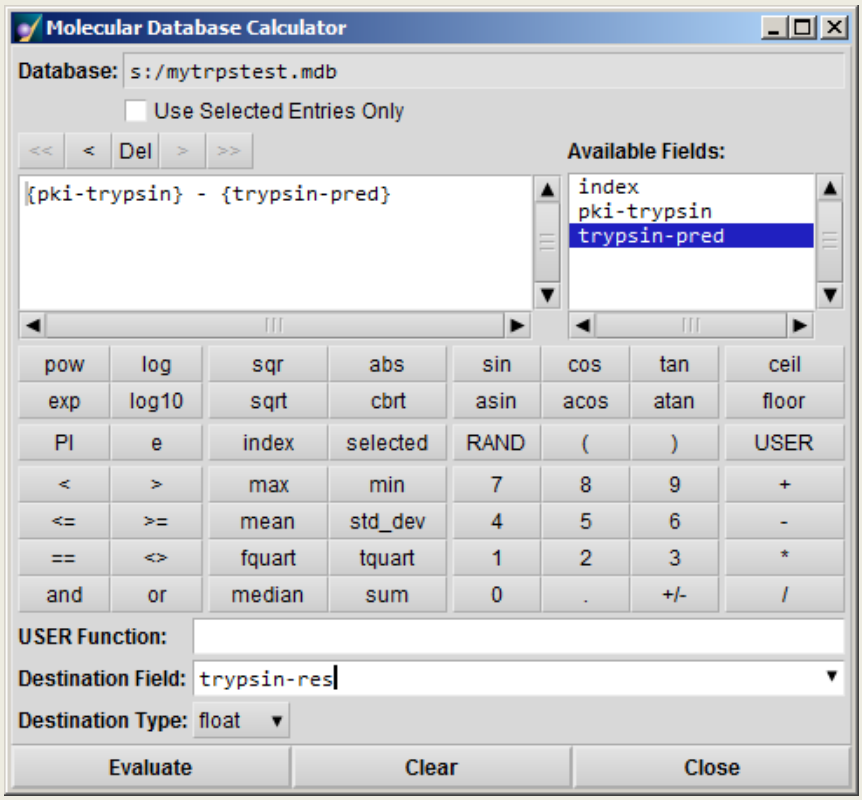


   a. Enter the name of the **Model File**: `trypsinpred.fit`.
   b. Change the name of the database **Field** to: `trypsin-pred`. Predicted values will be written to this field.
   c. Press **OK** to evaluate the predicted values.

4. The residuals between the experimental pK_i values (`pki-trypsin`) for the entries in the test set and the predicted values (`trypsin-pred`) can be calculated using the Molecular Database Calculator:

   DBV | Compute | Calculator

   a. In the Available Fields area, double-click on **pki-trypsin**. The selected field appears in the equation area on the left of the panel.
   b. Click on the minus sign button.
   c. Double-click on the **tryps in-pred** field to insert it in the equation.
   d. As **Destination Field**, change the default name to: **trypsin-res**.



e. Press Evaluate. The results, i.e. the residuals for the test entries, are written in a new field called **trypsin-res** in the `mytrpstest.mdb` database.
f. Close the Molecular Database Calculator.

5. Scroll right, to the end of the database, to see the **trypsin-res** column. For the purposes of this exercise, let us suppose that a deviation of 1 pK_i unit or more from the experimental value is a measure of a poor fit. Examination of the residuals for the test molecules indicates that, for the molecules with index 77 and 86, the fit is inadequate.

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VI. Pruning the Descriptors

It is sometimes necessary to prune a set of descriptors in order to select the optimum set for the molecules under consideration.

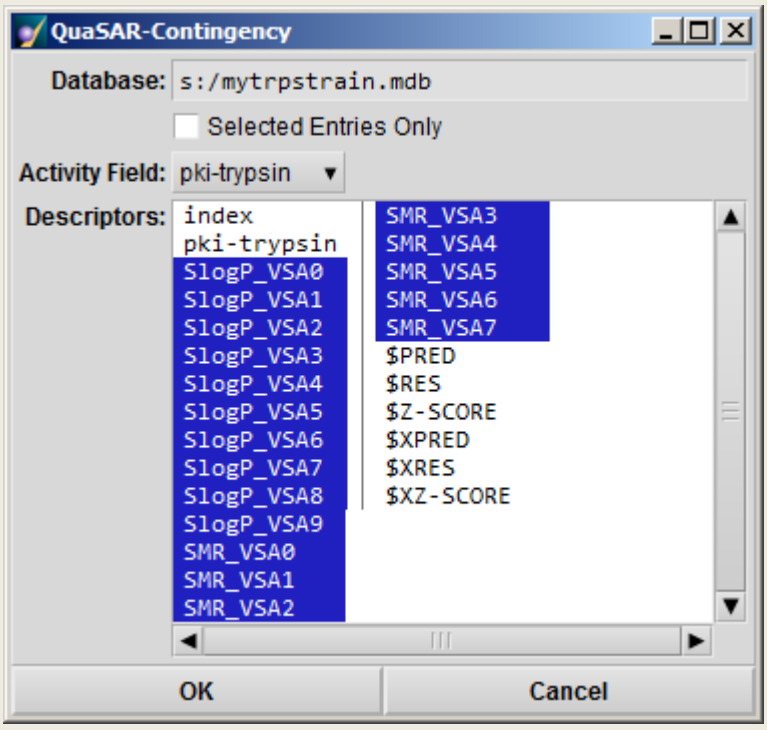
1. Return to the database which contains the descriptor values for the training set of molecules:

   DBV | File | Open | `mytrypstrain.mdb`

   Alternatively, you can use the **DBV** menu in the top right of the Database Viewer menu.

2. We will now use QuaSAR-Contingency, a statistical application in MOE designed to assist you in the selection of descriptors. It will suggest a set of descriptors that best describes the molecules in the training set.

   DBV | Compute | Descriptors | Contingency



   a. As **Activity Field**, select `pki-trypsin`.
   b. Select all the descriptors, from `SlogP_VSA0` to `SMR_VSA7`.
   c. Press **OK** to execute the contingency analysis. The results appear in an SVL Text Editor.

   Take some time to peruse the information given in this report. Recommended ranges for the indices appear at the top. These ranges help you to determine which descriptors are good and which are not. The results for each descriptor field are summarized on a separate line. The columns are the descriptor number, the contingency coefficient *C*, Cramer's *V*, the uncertainty coefficient *U* and the correlation coefficient R^2.

   Suggested descriptors for QSAR can be found at the bottom of the report. You may obtain a better model using QuaSAR-Model with these suggested descriptors.

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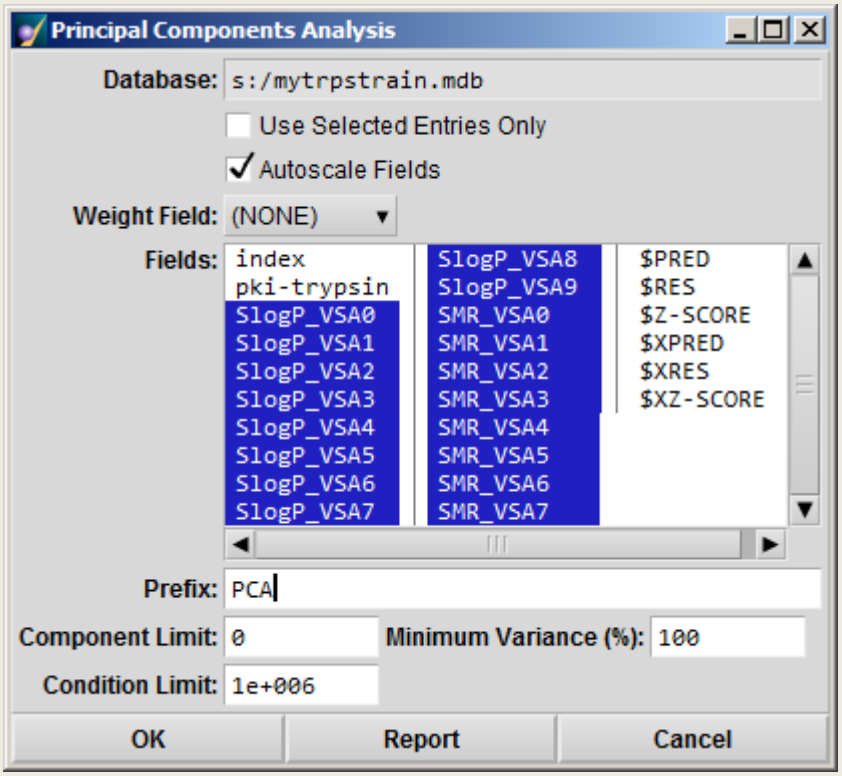
3. After performing the QSAR fit, it is recommended to analyze the results using Principal Components Analysis (PCA). The purpose of PCA is to reduce the dimensionality of set of molecular descriptors by linearly transforming the data. PCA is capable of calculating a new, often smaller, table of descriptors that are uncorrelated and normalized (mean 0 and variance 1).
4. Returning to `mytrpstrain.mdb`, choose:

   DBV | Compute | Descriptors | Principal Components



   a. Select the molecular descriptors `SlogP_VSA0` to `SMR_VSA7`.
   b. Set the **Minimum Variance** to 98. This is the minimum percentage of variance to retain in the final set of principal components. In other words, MOE will retain the number of principal components required to account for 98% of the variance.

      Leaving the Component Limit at its default value of zero means that there will be no limit to the number of components to retain.

   c. Press **Report**. The report appears in an SVL Text Editor. Save the report as `pca.rep` using **File | Save As** in the Text Editor.

      The columns in the table are:

      #PC — Principal component number.

      Deviation — Square root of the eigenvalue of the covariance matrix corresponding to the principal component, i.e. the standard deviation of the data along the

principal component vector.

Condition Condition number of the covariance matrix if the principal component list were terminated at that row.

%Var Percentage of the variance retained if the component list were truncated at that row.

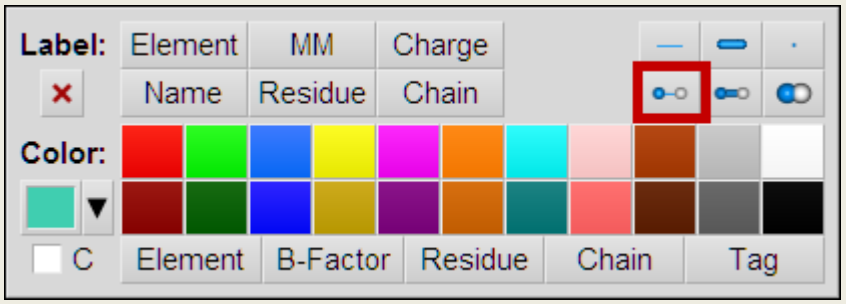
From the report, 12 principal components (PCA) are required to capture 98% of the variance in the data set. The actual mathematical form of each of the principal components is also given in the report.

d. Press **OK** to perform the principal components analysis.

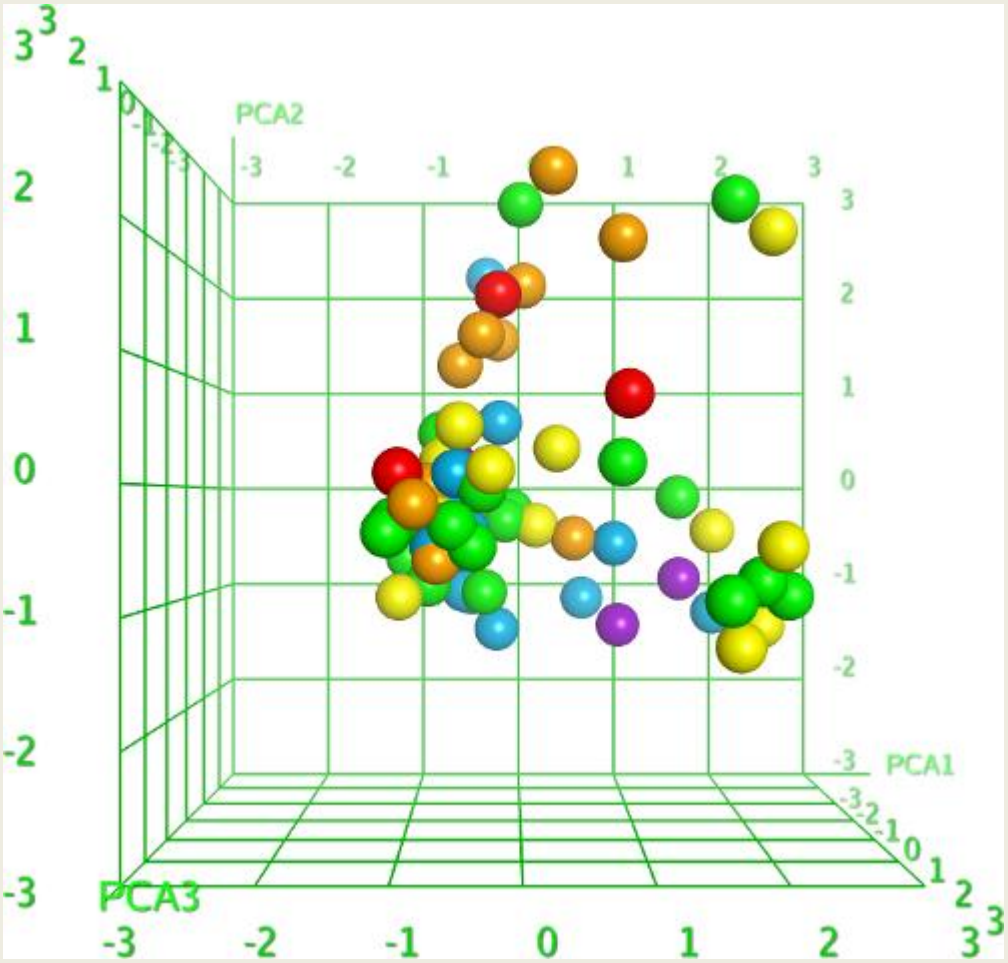
5. You can obtain a graphical display of the experimental activity of the inhibitors (`pki-trypsin`) with the first three principal components:

DBV | Compute | Analysis | 3D Plot

a. Set the **X Axis** to `PCA1`, **Y** to `PCA2` and **Z** to `PCA3`.
b. Toggle off Decorrelate Axes
c. Set the **Activity** to `pki-trypsin`.



d. Press **Plot**. The 3D scatter plot in the MOE Window is a visual representation of the molecules as described by the three selected Principal Components (PCA1, PCA2, PCA3). Each point in the MOE Window corresponds to a molecule and is colored according to the molecule's pK_i value. Leave the 3D Plot window open.
e. Render atoms in ball-and-line with MOE | Render | Atoms



f. As long as the 3D Plot window has been left open, selecting the points in the main MOE window will select the entry it represents in the Database Viewer. Additionally, clicking Browse in the 3D Plot panel will launch the Database Browser showing a 2D depiction of the currently selected molecule. Also notice that labelling the activity value is written into the plot atom's partial charge, which can be shown as a label from the MOE | Render | Atoms panel.

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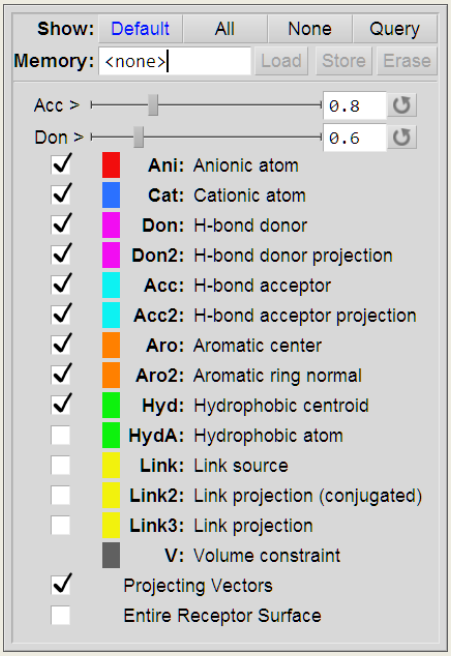
Pharmacophore Searching

Aim: Introduction to pharmacophore generation and pharmacophore search

A pharmacophore is a set of structural features in a ligand that are directly related to the ligand's recognition at a receptor site and its biological activity.



We will first align three active molecules, create a pharmacophore based on common superimposed features and then we will perform virtual screening to identify potential hits in a chemical database

To generate 3D coordinates in the database inhouse.mdb:

```
MOE | File | Open database.mdb
MOE DBV | Compute | Energy Minimize
```

To generate a set a pool of 3D conformers as in database.mdb:

```
MOE | File | Open database.mdb
Import Conformations     set up the conformer generation and the
                         database output name.mdb).
```

1. 3D Alignment of ligands:

```
MOE | File | Open ligands.mdb
```

In the MOE database viewer, select the most three active compounds (Ctrl and click left) and right click one of these and click **Copy Selected to MOE** in the popup menu. Close the **Database Viewer**.

```
MOE | Compute | Conformations | Flexible Alignment
```

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```
In the Flexible Alignment window, lower the iteration limit to 20 and
the Failure limit to 5. Do not change the other options. Start
calculation by pressing OK.
```

The calculation lasts for several minutes. A new database named flexalgn.mdb is created. It includes the possible alignments.

Open ligands_ali.mdb database and compare the overlay of compounds **1**, **2** and **3** in the different MOE-alignment solutions. Copy one alignment in MOE main GUI and close the all Database Viewer.

2. Pharmacophore query generation

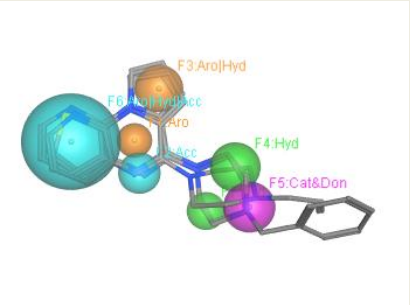
```
MOE | New | Pharmacophore Query
```

In the **Pharmacophore query editor** window, give a title to the Query (e.g. PPC413). It is possible to create the query either manually or automatically. Choose the second option by clicking on **Consensus...**.

In the **Pharmacophore Consensus** windows, select **MOE** as input, then click on **calculate**. A set of pharmacophore features is displayed into the MOE main GUI. Select three to five key features and click on **Load Selected**. Close the window.

In the **Pharmacophore query editor** window, **save** your query as PPC413.ph4

Close the window.



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3. Pharmacophore search

```
MOE DBV | File | Pharmacophore search
```

In the Query section of the **Pharmacophoric Search** window,

Browse… the PPC413.ph4 query. Click on **Search** to start the calculation.

Report results in Table .

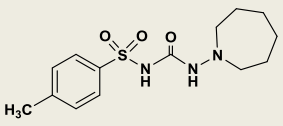
Flexible alignment

Aim: Look which the ligands are similar by determining which features they have in common

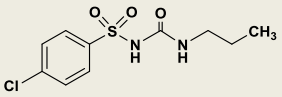
To perform a good alignment:

a. The strain energy of each molecule is small
b. Molecules have a similar shape
c. Molecules have similar LogP (octanol/water) values
d. Molecules have comparable molar refractivities
e. Aromatic atoms overlap
f. Hydrogen bond donors and acceptors overlap
g. Acidic groups and basic groups overlap
h. Atoms of similar partial charge overlap
i. Hydrophobic areas overlap

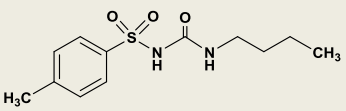
Examples:



Tolazamide



Chlorpropamide



Tolbutamide

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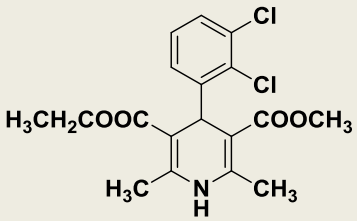
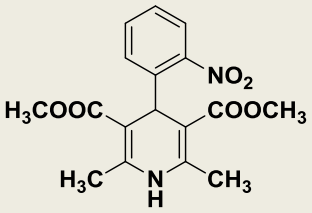
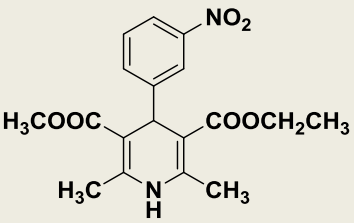


Felodipine



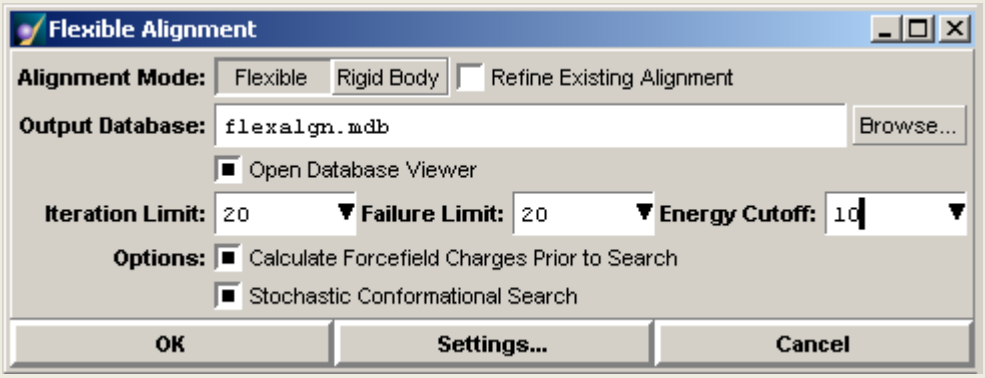
Nifedipine



Nitrendipine

How to apply:

4. **Draw the three drugs using MOE Builder**
5. **Compute >>> energy minimize**
6. **MOE | Compute | Conformations | Flexible Alignment** – mark on Refine the existing alignment



7. **Analyze the output by defining the similar features essential for drug activity**

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Molecular properties calculations

Aim: study of physicochemical properties of drugs and figuring out which parameters are good for activity

1. **Partition coefficient** (LogP values): Log of the octanol/water partition coefficient

LogP is one criterion used in medicinal chemistry to assess the druglikeness of a given molecule, and used to calculate Lipophilic efficiency, a function of potency and LogP that evaluate the quality of research compounds. For a given compound lipophilic efficiency is defined as the pIC50 (or pEC50) of interest minus the LogP of the compound.

Lipophilic Efficiency = pIC50 (or pEC50) – LogP

- The calculated value cLogP is often used instead of the measured LogP.
- LiPE is used to compare compounds of different potencies (pIC_{50}s) and lipophilicities (LogP). High potency (high value of pIC50) is a desirable attribute in drug candidates, as it reduces the risk of non-specific, off-target pharmacology at a given concentration. When associated with low clearance, high potency also allows for low total dose, which lowers the risk of idiosyncratic drug reaction.

2. **Drug solubility**; LogS

Solubility, the phenomenon of dissolution of solute in solvent to give a homogenous system, is one of the important parameters to achieve desired concentration of drug in systemic circulation for desired (anticipated) pharmacological response. Low aqueous solubility is the major problem encountered with formulation development of new chemical entities as well as for the generic development. More than 40% NCEs (new chemical entities) developed in pharmaceutical industry are practically insoluble in water. Solubility

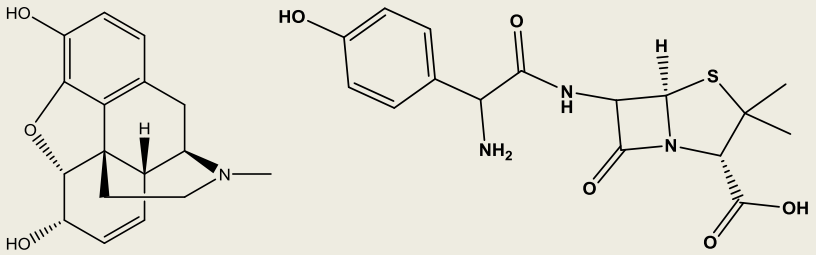
is a major challenge for formulation scientist. Any drug to be absorbed must be present in the form of solution at the site of absorption.

3. **Acid dissociation constant**; pKa

pKa is the negative logarithm (base-10) of the acid dissociation constant (Ka) of a solution. pKa = -logKa

The lower the pKa value, the stronger the acid.

- For example, the pKa of acetic acid is 4.8, while the pKa of lactic acid is 3.8. Using the pKa values, one can see lactic acid is a stronger acid than acetic acid.
- Ionizable groups indicates acid/base strength like OH phenolic, COOH, Amines.



4. **Lipinski's rule of 5 = Pfizer's rule of five = rule of 5:**

Used to evaluate druglikeness or determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans and a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism, and excretion ("ADME"). However, the rule does not predict if a compound is pharmacologically active.

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Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

1. Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
2. Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
3. A molecular mass less than 500 daltons
4. An octanol-water partition coefficient log *P* not greater than 5

QSAR and molecular descriptors

Parameter	*Symbol*
Hydrophobic parameters	
Partition coefficient	log *P*
Substituent constant	π
Hydrophobic fragmental constant	*f*, *f'*
Distribution coefficient	log *D*
Apparent partition coefficient (fixed pH)	log *P'*, log P_{app}
Capacity factor in HPLC	log *k*, log k_w
Solubility parameter	log S
Electronic descriptors	
Hammett constants	$\sigma, \sigma^-, \sigma^+$
Taft's inductive (polar) constants	σ^*, σ_I
Swain and Lupton field parameter	*F*
Swain and Lupton resonance parameter	*R*
Ionization constant	pK_a, ΔpK_a
Chemical shifts (^{13}C and ^{1}H)	δ
Steric descriptors	
Taft's steric parameter	E_s
Molar volume	*MV*
Molecular weight	*MW*
Van der Waals radius	*r*
Van der Waals volume	V_w
Molar refractivity	*MR*
Parachor	P_r
STERMOL parameters	L, B_i

1. Hydrophobic parameters

Clearly absorption and distribution processes in biological systems are determined by the **hydrophilic or hydrophobic** properties of molecules, for which the **partition coefficient, P**, **of a molecule** is used. **P** is defined by:

$$\mathbf{P} = \frac{[\text{drug}]_{\text{octanol}}}{[\text{drug}]_{\text{water}}}$$

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By using a logarithmic relationship, P becomes an additive property:

$$\mathbf{logP} = \log[\text{drug}]_{\text{octanol}} - \log[\text{drug}]_{\text{water}}$$

The contribution of a substituent, X, to the logP of a molecule is defined by

$$\pi_X = \log P_{\substack{\text{substituted}\\\text{compound}}} - \log P_{\substack{\text{parent}\\\text{compound}}}$$

$$= \log \frac{P_{RX}}{P_{RH}}$$

π is the hydrophobic parameter for a specific substituent. Extensive measurements and use of theoretical correlations have resulted in a large number of tabulated **logP** and π values. Typical values for π for different substituents are:

Hydrophobic		Hydrophilic	
Substituent	$\pi > 0$	**Substituent**	$\pi < 0$
$-CH_3$	0.56	**$-NO_2$**	-0.28
$-C(CH_3)_3$	1.98	**-OH**	-0.67
$-C_6H_5$	1.96	**$-CO_2H$**	-0.32
$-C_6H_{11}$	2.51	**$-NH_2$**	-1.23
$-CF_3$	0.88	**-CHO**	-0.65

The usefulness of π values is to estimate **logP** of a substituted compound, given the logP of a parent molecule. For example,

$$\mathbf{LogP}_{\text{phenol}} = \log\mathbf{P}_{\text{benzene}} + \pi_{OH} = 2.13 + (-0.67) = 1.46$$

$$\mathbf{P} = \frac{[\text{drug}]_{\text{octanol}}}{[\text{drug}]_{\text{water}}} = \frac{2,884}{100}$$

$$\text{Log}\mathbf{P}_{\text{toluene}} = \log\mathbf{P}_{\text{benzene}} + \pi_{CH3} = 2.13 + (0.56) = 2.69$$

$$\mathbf{P} = \frac{[\text{drug}]_{\text{octanol}}}{[\text{drug}]_{\text{water}}} = \frac{48,900}{100}$$

$$\log\mathbf{P}_{\text{PABA}} = \log\mathbf{P}_{\text{benzene}} + \pi_{CO2H} + \pi_{NH2} = 2.13 + (-0.32) + (-1.23) = 0.58$$

$$\mathbf{P} = \frac{[\text{drug}]_{\text{octanol}}}{[\text{drug}]_{\text{water}}} = \frac{380}{100}$$

Examples

Log P values are dependent on the experimental method, as shown for the recorded values for phenobarbital, $C_{12}H_{12}N_2O_3$. Where a molecule possesses one or more **ionizable groups**, each of which makes a contribution to the overall partition coefficient, then **logD** would need to be used particularly at pH values far from that of the neutral species of interest. Based on correlations between experimentally measured and theoretical values, contributions to logP have been calculated **for molecular fragments**, enabling an estimate to be made for the **logP** of a molecule **based on the structural formula**.

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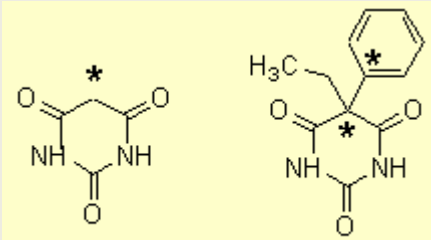


For example, for phenobarbital, a logP of 1.71 was *estimated* using the following **fragmental constants**:

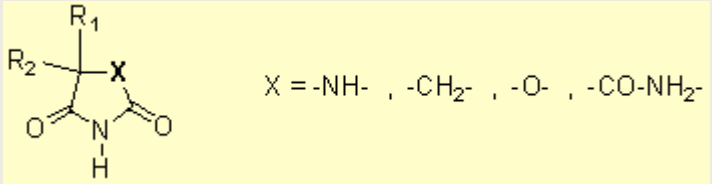
-CH=(aryl)	0.37	-CH=(aryl*)	-0.08
>C<(*)	-0.22	$-CH_2-$	0.53
$-CH_3$	0.91	barbiturate	-2.65
Interactions between aliphatic and aryl systems:			0.35
Interactions within barbiturate system:			0.67

logP = 5(0.37) -0.08 -0.22 + 0.53 + 0.91 -2.65 + 2(0.35) + 0.67 = 1.71

Note that the -C=O and -NH- fragments of the barbituric acid portion have a *combined* effect on logP when closely associated, as in these molecules, that is different from their values in isolation. As a result, barbituric acid has a calculated logP of -1.41. Similarly, the starred atoms (*) differ from normal sp^3 and sp^2 atoms in their intramolecular interactions depending on whether they are in barbituric acid, or phenobarbital, where extra effects occur.

Anticonvulsant activity of a diverse series of drugs has been shown to be related to logP by the equation:

$$\log (1/C) = 0.73\,\mathbf{logP} + 2.5$$



This implies that anticonvulsant activity would increase *indefinitely* by increasing the lipophilicity of the drugs. Clearly, however, there must be a limit to this increase: lipophilic molecules could become trapped in fat depots, thereby decreasing their availability and activity.

Extension of the above study to include more lipophilic molecules supports this, giving overall a parabolic relationship. By differentiation, this gives an optimum log P value of approximately 1.75, a value typical for most CNS-active drugs.

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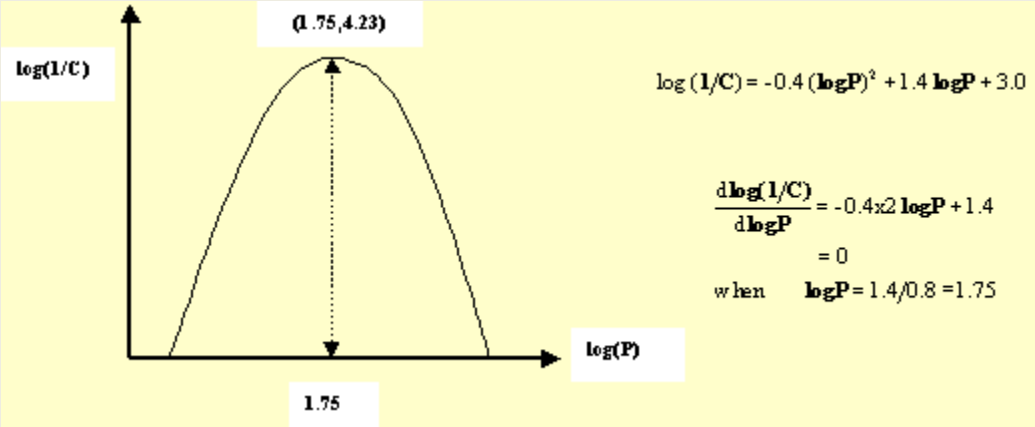
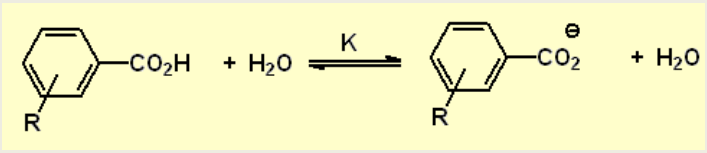


2. Electronic parameters

Electronic properties were initially developed from a consideration of substituent effects in aromatic compounds. For example, the **dissociation constants of substituted benzoic acids** ($Kx10^5$ at 25^oC) were used by Hammett



R	H	CH_3	OCH_3	F	Cl	NO_2
ortho	6.27	12.3	8.06	54.1	11.4	671
meta	6.27	5.35	8.17	13.6	14.8	32.1
para	6.27	4.24	3.38	7.22	10.5	37.0

What is the effect of R on the acidity of the acids?

acid form is stabilized if R is **electron donating**, therefore, the **equilibrium lies to the left**

anion form is stabilized if R is **electron-withdrawing,** therefore, the **equilibrium lies to the right**

Electronic properties can be quantified by K_a, as follows:

$$\log K_{a\,(\text{substituted acid})} - \log K_{a\,(\text{unsubstituted acid})} = \log K_{a\,(RX)} - \log K_{a\,(RH)}$$
$$= \log \frac{K_{a\,(RX)}}{K_{a\,(RH)}}$$
$$= \sigma$$

where σ **is the substituent constant** for a given group, R, and its position, and K_a are acid dissociation constants. It is possible to have σ_o, σ_m and σ_p values. Typical values of the substituent constants, σ_m and σ_p are shown in the following table.

Substituent			Substituent		
	Meta	*Para*		*Meta*	*Para*
O	-0.708	-1.00	F	+0.337	+0.062

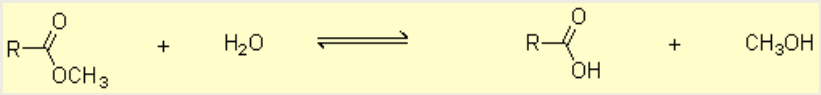
OH	+0.121	-0.37	Cl	+0.373	+0.227
OCH_3	+0.115	-0.268	CO_2H	+0.355	+0.406
NH_2	-0.161	-0.660	$COCH_3$	+0.376	+0.502
CH_3	-0.069	-0.170	CF_3	+0.43	+0.54
$(CH_3)_3Si$	-0.121	-0.072	SO_2Ph	+0.61	+0.70
C_6H_5	+0.06	-0.01	NO_2	+0.710	+0.778
H	0.000	0.000	$^+N(CH_3)_3$	+0.88	+0.82
SH	+0.25	+0.15	N_2^+	+1.76	+1.91
SCH_3	+0.15	0.00	$^+S(CH_3)_2$	+1.00	+0.90

σis transferable to many reactions involving benzene and other aromatic species, leading to a generalized form of the equation known as the **Hammett equation**:

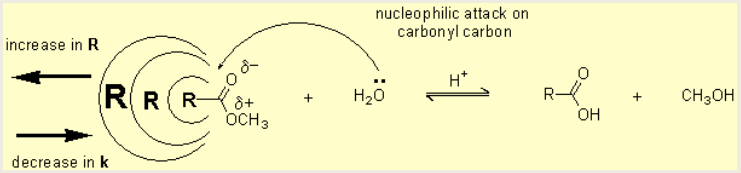
$$\rho\sigma = \log K_{a(R)} - \log K_{a(H)}$$

3. Steric parameters

Taft quantified the **steric** (spatial) effects using the hydrolysis of esters:



Here, the size of R affects the rate of reaction by *blocking nucleophilic attack by water*.



In this case, the steric effects were quantified by the **Taft parameter** $\mathbf{E_s}$**:**

$$E_S = \log k_{RCO_2CH_3} - \log k_{CH_3CO_2CH_3}$$
$$= \log \frac{k_{RCO_2CH_3}}{k_{CH_3CO_2CH_3}}$$

k is the rate constant for ester hydrolysis. This expression is analogous to the Hammett equation.

Compare some extreme values:

	H	1.24 +ve value: **little steric resistance** to hydrolysis
	Me	0.00 the reference substituent in the Taft equation
	t-Bu	-2.78 -ve value: **large resistance** to hydrolysis

Typical $\mathbf{E_s}$ values are:

E_s Values for Various Substituents

H	Me	Pr	*t*-Bu	F	Cl	Br	OH	SH	NO_2	C_6H_5	CN	NH_2
0.0	-1.24	-1.60	-2.78	-0.46	-0.97	-1.16	-0.55	-1.07	-2.52	-3.82	-0.51	-0.61

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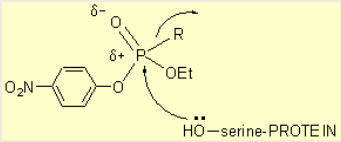
Note: H is usually used as the reference substituent (E_s^0), but sometimes when another group, such as methyl (Me) is used as the reference, as in the chemical equation above, the value becomes 1.24.
As was the case for σ , E_s may be used in other chemical reactions and to explain biological activities, for example the hydrolysis of inhibitors of acetylcholine esterase.



Organophosphates must be hydrolysed to be active and it is observed that their biological activity is directly related to the Taft steric parameter $\mathbf{E_s}$ for the substituent R by the equation:

$$\log(1/C) = 2.58\,E_s + 7.94$$

- See if you can deduce correlations for the following two sets of data.

(i) Isonarcotic Activity in Tadpoles					
Esters, Ketones, and Ethers			**Alcohols**		
Compound	**log (1/C)**	**log P**	**Compound**	**log (1/C)**	**log P**
CH_3COCH_3	0.65	-0.73	**CH_3OH**	0.30	-1.27
$CH_3CO_2CH_3$	1.10	-0.38	**C_2H_5OH**	0.50	-0.75
$C_2H_5COCH_3$	1.10	-0.27	**$(CH_3)_2CHOH$**	0.90	-0.36
$HCO_2C_2H_5$	1.20	-0.38	**$(CH_3)_3COH$**	0.90	0.07
$C_2H_5COC_2H_5$	1.20	0.59	**$CH_3CH_2CH_2OH$**	1.00	-0.23
$CH_3CO_2C_2H_5$	1.50	0.14	**$(CH_3)_2C(C_2H_5)OH$**	1.20	0.59
$C_2H_5COC_2H_5$	1.50	0.31	**$CH_3(CH_2)_3OH$**	1.40	0.29
$CH_3CH_2CH_2COCH_3$	1.70	0.31	**$(CH_3)_2CHCH_2OH$**	1.40	0.16
$CH_3CO_2CH_2C_2H_5$	2.00	0.66	**$CH_3(CH_2)_4OH$**	1.60	0.81
$C_2H_5CO_2C_2H_5$	2.00	0.66			
$(CH_3)_2CHCO_2C_2H_5$	2.20	1.05			

(ii) Growth Inhibition for Hamster Ovary Cells [redacted]

R	IC_{50}, mM	$Log1/IC_{50}$	σ	π
H	1.2	-0.079	0	0.00
m-NO_2	96	-1.982	0.71	-0.28
p-SO_2Me	174	-2.241	0.72	-1.63
m-SO_2Me	76	-1.881	0.63	-1.63
p-$CONMe_2$	14.6	-1.164	0.36	-0.71
m-$CONMe_2$	7.6	-0.881	0.35	-0.71
p-SMe	1.62	-0.210	0	0.61
p-CH_3	0.4	0.398	-0.14	0.56

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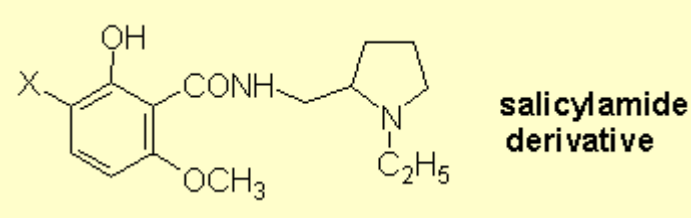
m-CH_3	1.2	-0.079	-0.06	0.56
p-OMe	0.45	0.347	-0.28	-0.02
m-OMe	2.7	-0.431	0.1	-0.02
p-NH_2	0.071	1.149	-0.66	-1.23
m-NH_2	0.38	0.420	-0.16	-1.23

• Salicylamides**.** Define the terms logP and π, and briefly describe how they are obtained. The influence of the aromatic substituent X in a series of monosubstituted salicylamides on their ability to displace [^{3}H]-spiperone (measured by IC_{50} values in nM) leads to the equation



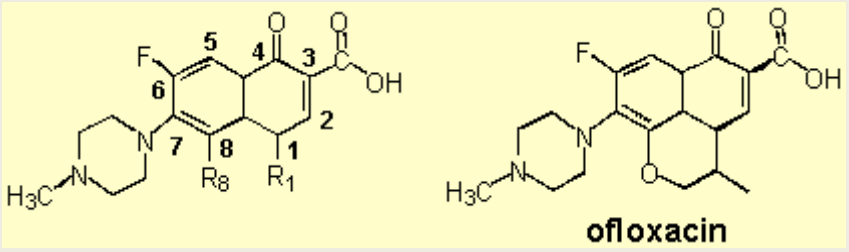
$$\log(1/IC_{50}) = 1.28\,\pi - 0.52\,\pi^2 + 1.54$$

n = 12 r^2 = 0.94 s = 0.40 F = 19.9

From the equation and the table below, what is the best value for π, the corresponding IC_{50} value, and best substituent, X?

Substituent	H	F	Cl	Br	I	NO_2	CH_3	C_2H_5	C_3H_5	C_3H_7
π	0.00	0.14	0.71	0.86	1.12	-0.28	0.56	1.02	1.14	1.55

• **Fluoroquinolone antibacterials**. In QSAR studies of fluoroquinolone antibacterial agents,



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the following two equations were used to determine the best substituents for positions **1** and **8:**

for position **1** : $\log (1/MIC) = -0.49 (L_1)^2 + 4.10 (L_1) - 2.00$

for position **8** : $\log (1/MIC) = -1.00 (B_4)^2 + 3.73 (B_4) + 1.3$

(a) Define the parameters L_1, B_4 and MIC.

(b) From the equations

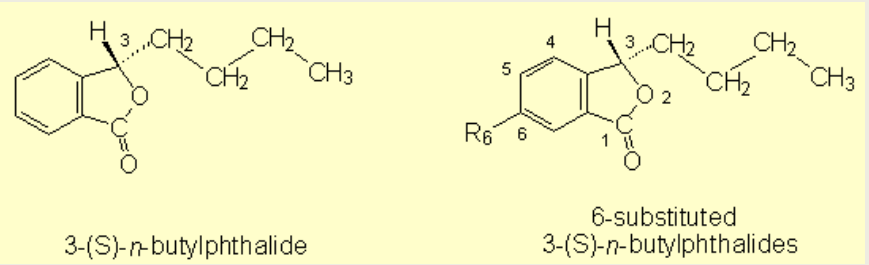
- determine the optimal values for L_1 and B_4. Use the table of L_1 and B_4 values given below to select the best groups for positions **1** and **8**.
- explain how this information contributed to the design of ofloxacin.

(c) Briefly describe the mode of action of the fluoroquinolone antibacterial agents and suggest how the QSAR results support this mechanism. (See T. Rosen's work)

Table of L_1 and B_4 values

Substituents	H	F	Cl	Br	I	Me	Et	*i*-Pr	*t*-Bu	*i*-Pentyl
L_1	2.06	2.65	3.52	3.83	4.23	3.00	4.11	4.11	4.11	4.73
B_4	1.00	1.35	1.80	1.95	2.15	2.04	2.97	3.16	2.97	3.99

• **Phthalides from Celery**. Since antiquity, celery extracts have been used in Chinese folk medicine to treat hypertension. More recently, investigations have also shown that celery seeds contain an oily liquid, 3-(S)-*n*-butylphthalide, which also has potential for development as an anti-epileptic agent.



QSAR analysis of a set of 6-substituted analogues of 3-(S)-*n*-butylphthalide gave the following Hansch equation. In the equation, ED_{50} (mol kg^{-1}) is the effective dose to protect mice against spasms induced by maximum electric shock.

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$$\log \frac{1}{ED_{50}} = 0.74 \log P - 0.13 (\log P)^2 + 0.35\ MR_6 + 2.23$$

$$n = 24 \quad r = 0.91 \quad s = 0.15 \quad F = 33.12$$

(a) Define the terms log P and MR_6. How does log P differ from the parameter, π ?

(b) Comment briefly on the significance of the values of n, r, s and F. How valid is this equation?

(c) Determine the best combination of values for log P and MR_6.

(d) From the table given below, what is the best substituent for the 6-position?

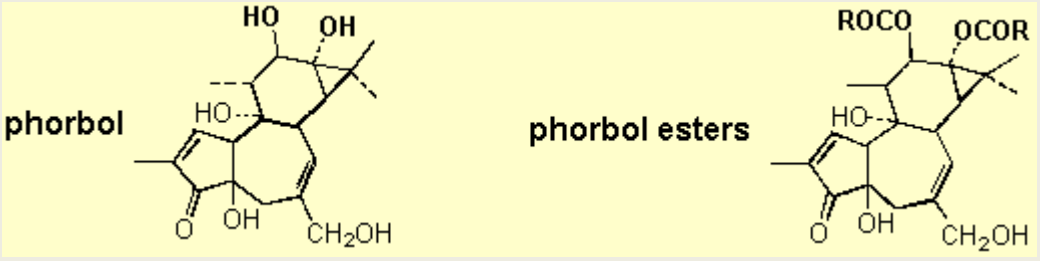
- Comment on the nature of the substituent you have selected in relation to the overall physicochemical properties of the compound.

(e) Calculate the corresponding ED_{50} for your 6-substituted 3-(S)-*n*-butylphthalide.

(f) The 6-amino (6-NH_2-) analogue, has a log $\frac{1}{ED_{50}}$ of 4.40. Compare this value with your ED_{50} and offer a possible explanation for any difference.

R_6	H	OH	Cl	Br	NO_2	CN	$CONH_2$
logP	3.12	3.12	3.53	4.24	2.92	2.39	2.25
MR_6	0.10	0.29	0.60	0.80	0.74	0.63	0.98

- **Phorbol Esters**. Two researchers studied the irritant properties of a series of phorbol esters.



They plotted the same data for concentration, C, to produce skin irritancy in the mouse ear test *versus* log P values, but fitted the two different curves, A and B. The statistics for each curve-fitting are given under the plots.

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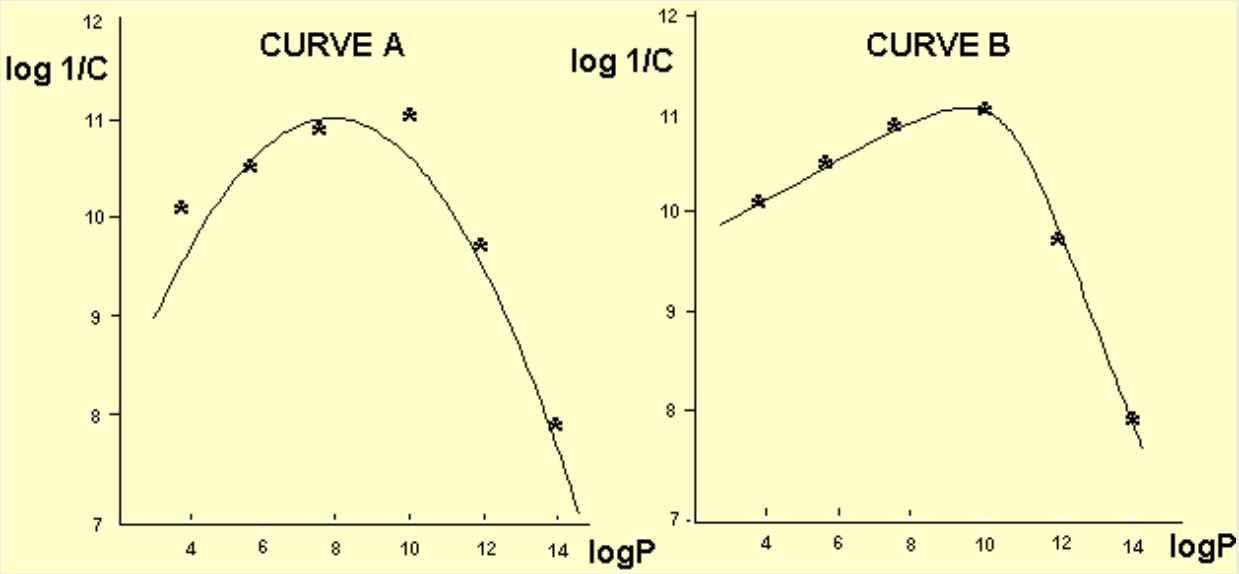

Curve A statistics	Curve B statistics
n = 6; r = 0.98; s = 0.32; F = 32.39	n = 6; r = 1.00; s = 0.04; F = 1,389.47

(a) Define log P and π.

(b) Decide which of the two plots gives the best fit to the data, giving reasons. From the curve you have chosen, what is the best value for log P and for C?

(c) From the table below of πvalues of alkyl groups, calculate which group gives the best activity for phorbol esters. Log P of the parent molecule, phorbol, is -0.12.

R	ethyl(C_2)	butyl(C_4)	hexyl(C_6)	octyl(C_8)	decyl(C_{10})	dodecyl(C_{12})	tetradecyl(C_{14})
π	0.87	1.93	3.00	4.06	5.12	6.18	7.25

(d) Give an explanation for the trend shown by the curve shapes above in terms of the structures and physicochemical properties of the phorbol esters.

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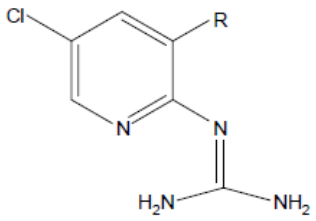
Lab#5

Molecular database and QSAR analysis

- Task: building the molecular database using software, by creation of fields and adding compounds in them.
- Database will be built using the down **compounds.** QSAR study of substituted 2-pyridinyl guanidines as selective urokinase-type plasminogen activator (uPA) inhibitors

Table II. Structure, biological activity and physicochemical descriptors of series 2



S.No	R	BA (μM)	−logBA	chi1v_C	b_double	E_sol	PMI − Z	logP (o/w)
1	(E)-CH=CHCO$_2$H	1.5	5.8239	2.0267	3	−15.3085	1328.947	1.04
2	(E)-CH=CHCONHMe	1.28	5.8928	2.0267	3	−15.1895	1889.878	0.662
3	E)-CH=CHCO(1-morpholino)	2.1	5.6778	3.0267	3	−15.4765	361.3822	0.425
4	(E)-CH=CHCONHCH$_2$Ph	0.77	6.1135	4.2909	3	−12.4917	1392.558	2.45
5	(E)-CH=CHCON(Me)CH$_2$Ph	2	5.6990	4.2909	3	−6.5011	2556.398	2.647
6	CH$_2$CH$_2$CO$_2$H	9.31	5.0311	2.3231	2	−14.8113	638.4941	0.572
7	OCH$_2$CO$_2$H	90.2	4.0448	1.4696	2	−13.5755	609.7509	0.18
8	OCH$_2$CONHCH$_2$Ph	16.1	4.7932	3.7338	2	−9.0434	353.2897	1.59
9	OCH$_2$CH$_2$OCH$_3$	16.4	4.7852	1.6160	1	−11.0577	1442.224	0.345
10	C$_6$H$_5$	166.3	3.7791	3.2767	1	−9.12613	939.6092	2.679
11	OC$_6$H$_5$	2.13	5.6716	3.0267	1	−12.9468	484.1166	2.329
12	OCH$_2$C$_6$H$_5$	0.92	6.0362	3.3803	1	−13.5082	2463.676	2.463
13	C≡CC$_6$H$_5$	0.77	6.1135	3.7767	1	−14.6776	1390.561	3.425
14	(E)-CH=CHC$_6$H$_5$	0.55	6.2596	3.9374	2	−13.2539	1335.126	3.323
15	(E)-CH=CHC$_6$H$_{11}$	0.73	6.1367	4.8879	2	−10.8807	118.8222	3.338
16	(E)-CH=CH(2-pyridinyl)	1.6	5.7959	3.3154	2	−13.3939	1285.165	2.052
17	(E)-CH=CH(4-C$_6$H$_4$OMe)	0.69	6.1611	3.8481	2	−12.8072	1330.748	3.279
18	E)-CH=CH[(3,4-OCH$_2$O)C$_6$H$_4$]	0.49	6.3098	3.7647	2	−14.2263	2392.332	3.028
19	(E)-CH=CH(3-C$_6$H$_4$CO$_2$H)	0.17	6.7695	4.0981	3	−14.1573	2820.975	3.037

$$-\log BA = 0.544(\pm 0.164)chi1v\_C - 0.156(\pm 0.069)E\_SOL + 0.0003(\pm 0.0001)PMI - Z + 1.505(\pm 1.220)$$

$$-\log BA = 0.419(\pm 0.099)\log P(o/w) - 0.110(\pm 0.045)E\_Sol + 0.272(\pm 0.139)b\_double + 2.935(\pm 0.748) \quad (3)$$

Practical Pharmaceutical Chemistry III

Substituents	NH_2	OH	CN	NO_2	H	F	SO_2Ph	OEt	CH_3	Cl	CF_3	$N(Et)_2$
π	-1.23	-0.67	-0.57	-0.28	0.0	0.14	0.270	0.38	0.56	0.71	0.88	1.18
σ	-0.66	-0.37	0.66	0.78	0.0	0.06	0.70	-0.24	-0.17	0.23	0.54	-0.90

- Calculate Lipinski's rule for all.
- Which compound is polar, and which is not?
- Measure different molecular descriptors that could describe the compounds.

$$\text{Log } 1/C = -0.31\pi^2 + 0.14\pi + 1.29\,\sigma + 4.56$$

$$n = 10 \quad r = 0.87 \quad s = 0.47$$

C is the ED_{50} concentration (moles per kilogram), in the anti-pentylenetetrazol induced seizure test in mice, a measure of anticonvulsant activity.

(a) Define π and σ.

(b) Comment briefly on the validity of the equation.

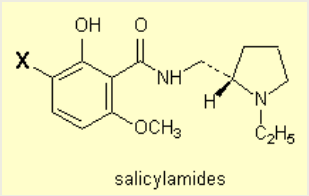
(c) Determine the best values for π and σ that give maximum anticonvulsant activity.

(d) From the table below, which substituent is the best choice to give this maximum? What physicochemical properties does the substituent have?

(e) If log P is 2.11 for the unsubstituted 1,4-benzodiazepinone, what is the log P for your substituted 1,4-benzodiazepinone?

- **Salicylamides**. A series of mono-substituted salicylamides was investigated to determine the influence of the aromatic substituent, X, on their ability to displace ^{3}H-spiperone from dopamine D_2 receptors *in vitro*. Analysis of the resulting data gave the following Hansch equation:

$$\log \frac{1}{IC_{50}} = 1.28\pi - 0.52\pi^2 - 0.69\sigma_m + 1.50$$



salicylamides

$n = 12$, $r^2 = 0.94$, $s = 0.40$, $F = 19.9$

(a) How valid is the equation? Briefly describe how this type of equation is obtained.

(b) What are the best values for the substituent parameters, π and σ_m?

(c) From the table of substituents, what is the best substituent for X?

(d) What is the IC_{50} for the salicylamide with the substituent you have chosen?

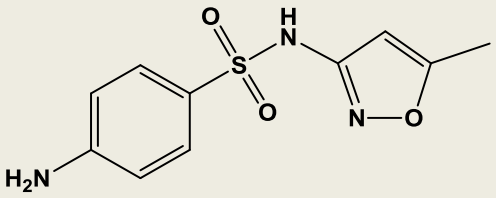
(e) Briefly comment on how the properties of this substituent contribute to the activity of the salicylamide.

(f) Sketch the general shape of the 3-D plot for the above equation.

X	$CONH_2$	NH_2	OH	CN	$NHCH_3$	NO_2	NHC_2H_5	NMe_2	NHC_3H_7	NHC_4H_9
π	-1.49	-1.23	-0.67	-0.57	-0.47	-0.28	0.08	0.18	0.62	1.16
σ_m	0.28	-0.16	0.12	0.56	-0.30	0.71	-0.24	-0.15	-0.24	-0.34

Practical Pharmaceutical Chemistry III

Assay of Sulpha Drugs (Sulphamethoxazole)



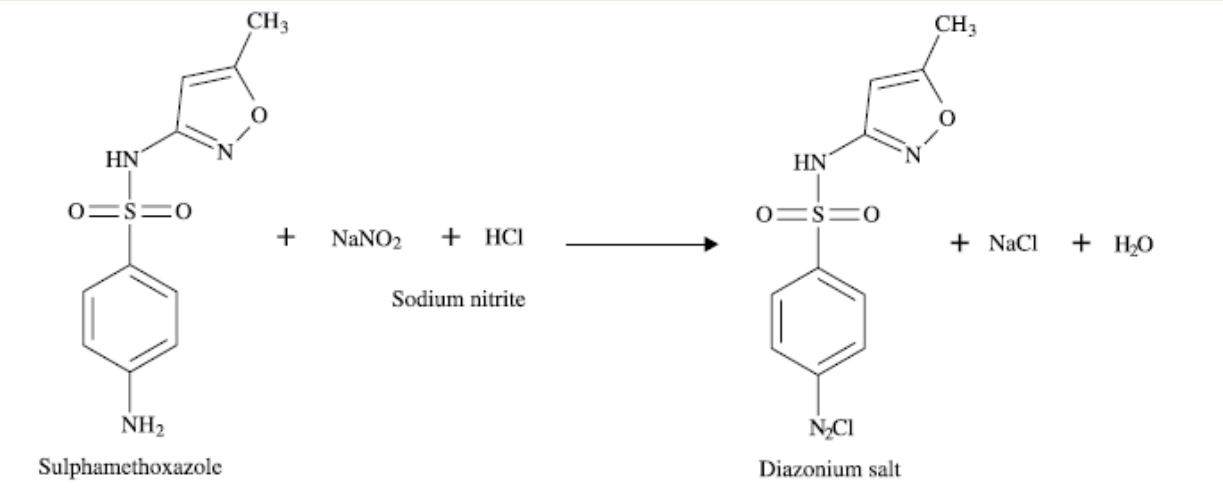
I. Assay using diazotization method

HCl, **$NaNO_2$** titrant, Starch-Iodide paper indicator

Yellow brown to blue color

Principle:

Sulphamethoxazole being a primary aromatic amine will undergo reaction with sodium nitrite in acidic condition (nitrous acid) to form a **diazonium salt**. Observation of end point depends on determination of small excess of **nitrous acid** which is determined by using the **starch-Iodide paper** as an external indicator. The **iodine** liberated reacts with starch to form a **blue** color.



$$HNO_2 + 2\,Na\,I \longrightarrow I_2 \xrightarrow{\text{Starch}} \text{Blue color}$$

Diazotization titrations method:

Weigh accurately about 0.5 g of Sulphamethoxazole sample and dissolve in 50 ml of 2M hydrochloric acid. Cool it in ice bath and add 2 gm of potassium iodide (KI). Dissolve and titrate slowly with 0.1 M **sodium nitrite** solution. The temperature should never go above 15ºC. continue titration until **yellow brown** color appear. The end point is determined by placing one small drop of solution is drawn from the conical flask with the help of glass rod on to a starch iodide paper. Appearance of **blue color** is the end point.

Calculation: Each ml of **0.1 M** $NaNO_2$ equivalent to **0.02528** gm of $C_{10}H_{11}N_3O_3S$.

Report: The given sample contains ---------- gm of Sulphamethoxazole.

Practical Pharmaceutical Chemistry III

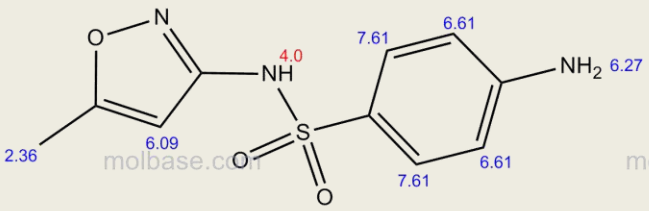
II. Chemical identification

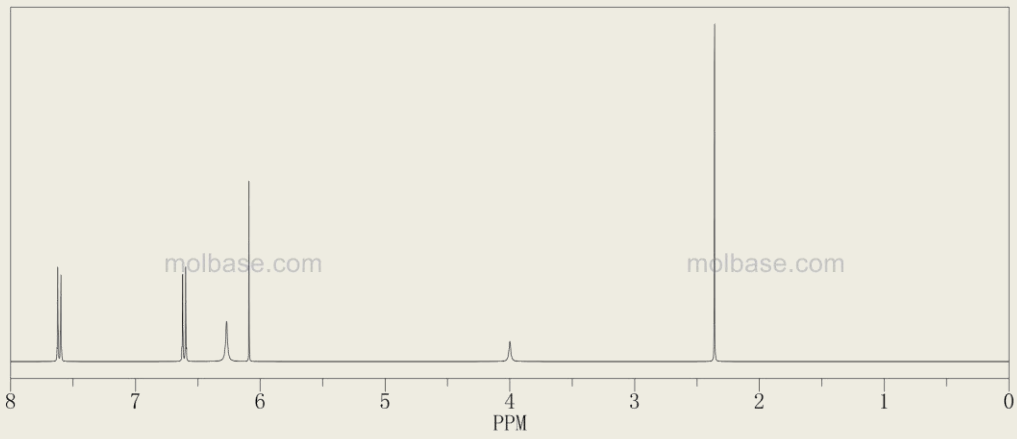
a. Azodye test

b. $FeCl_3$ test

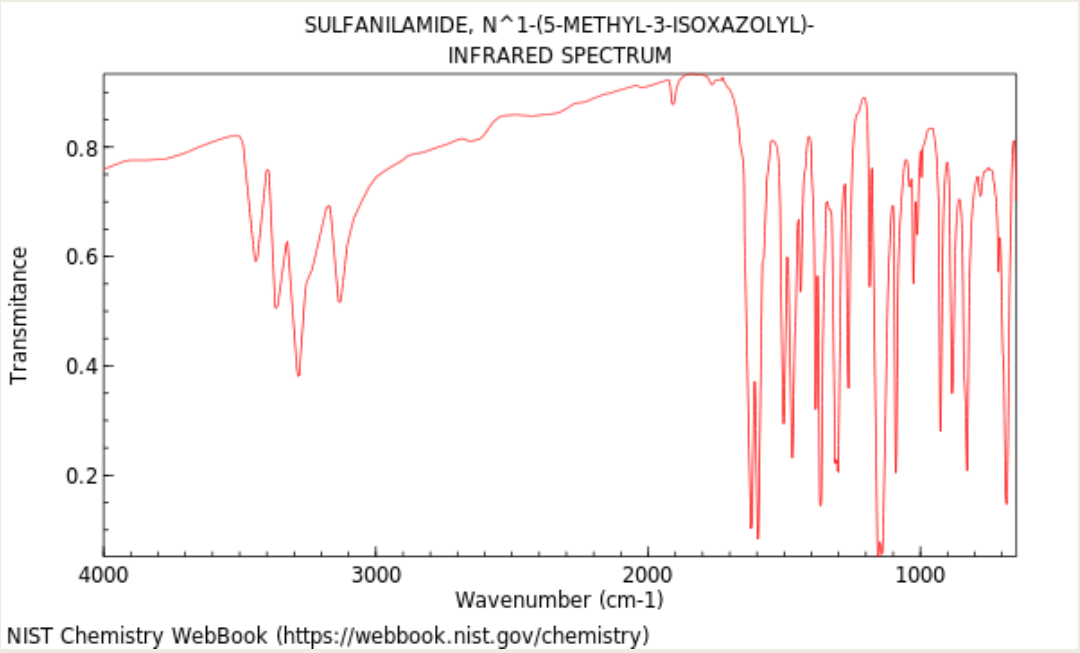
c. Spectroscopic identification

HNMR Spectrum





IR Spectrum

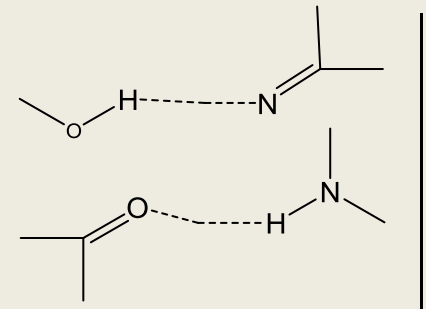


Practical Pharmaceutical Chemistry III

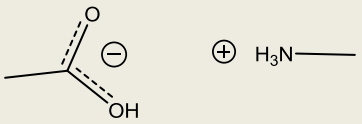
Ligand-target interaction

Predicting interactions between small molecules and proteins is a key element in the drug discovery process. In particular, several classes of proteins such as G-protein-coupled receptors (GPCR), enzymes and ion channels represent a large fraction of current drug targets and important targets for new drug development.

c. Types of ligand-target interactions:

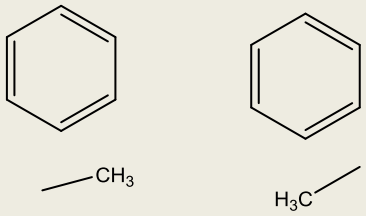


Hydrogen bonding interaction (ACCEPTOR and DONOR)

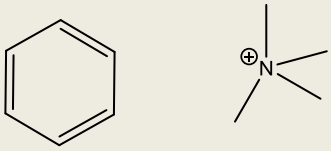


Ionic interaction (SALT BRIDGE)

Metal ion complex



Hydrophobic interaction (Aromatic stacking)



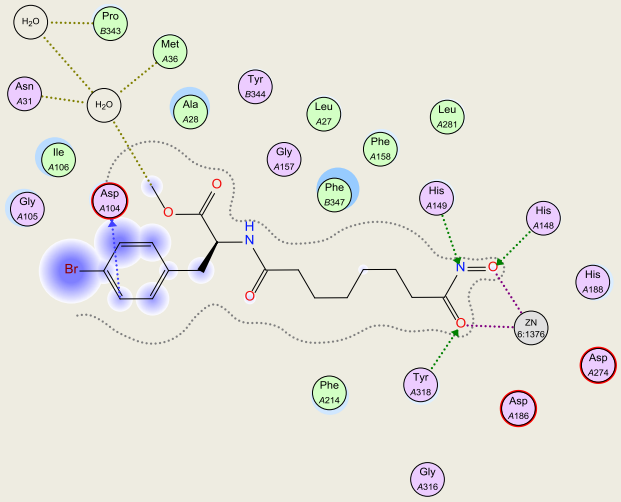
Cation--aromatic (bi) interaction

Practical Pharmaceutical Chemistry III

Structure of Mycobacterium tuberculosis CYP121 in complex with the antifungal drug fluconazole

d. Open the crystal structure of **CYP121** complex with fluconazole in MOE (2IJ7 PDB)
e. CLICK on the right corner SEQ
f. Analyze the structure through , how many amino acids there, drugs, number of H2O molecules, metals, ions, or others
g. MOE/Compute / ligand interaction
h. In the moe window of interaction /isolate to look deep and extract the binding information:

1. Binding pocket amino acids
2. Types of interaction
3. Fragments of ligand involved in the interaction



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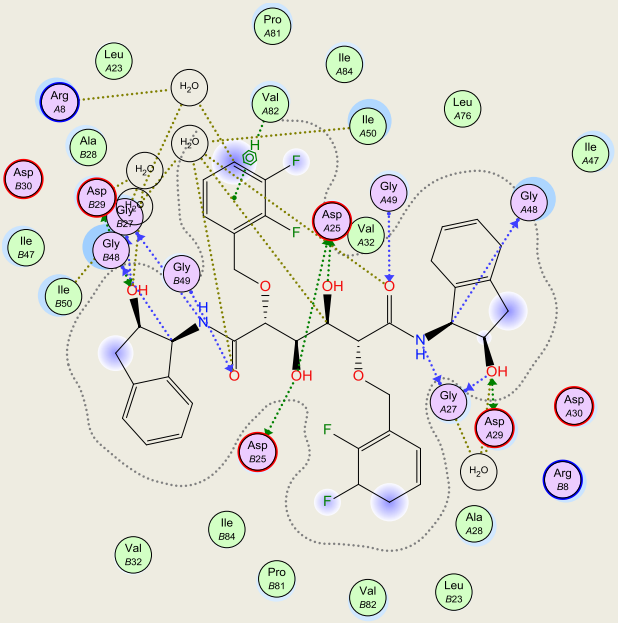

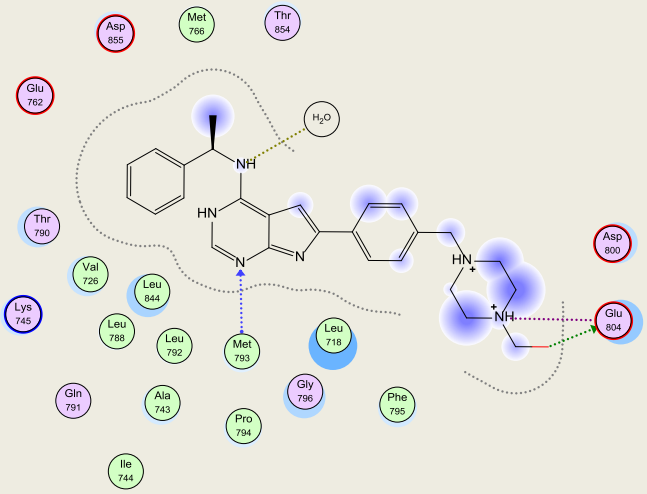

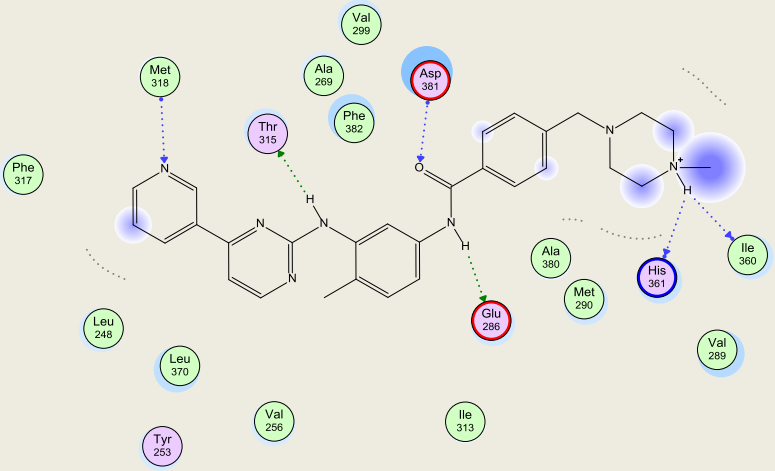

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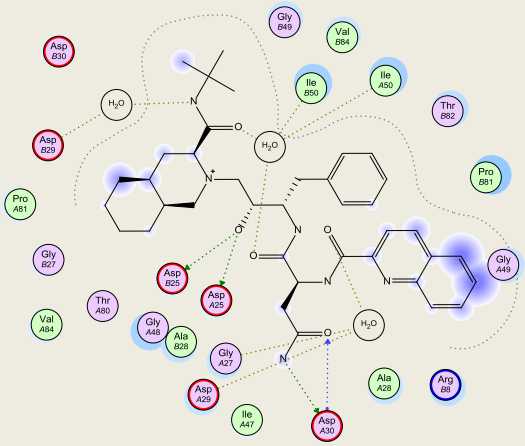

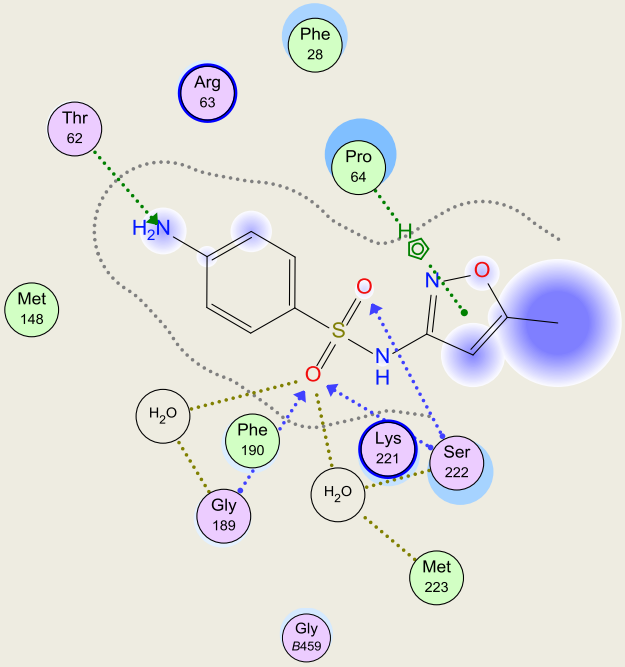

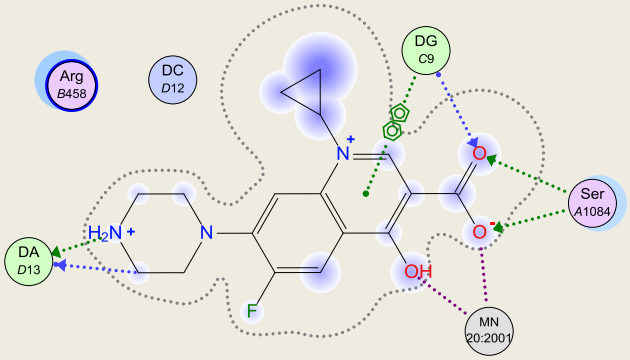
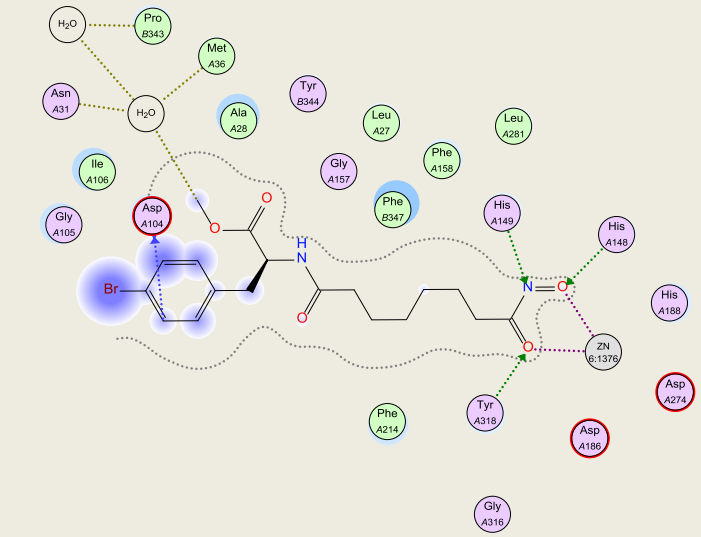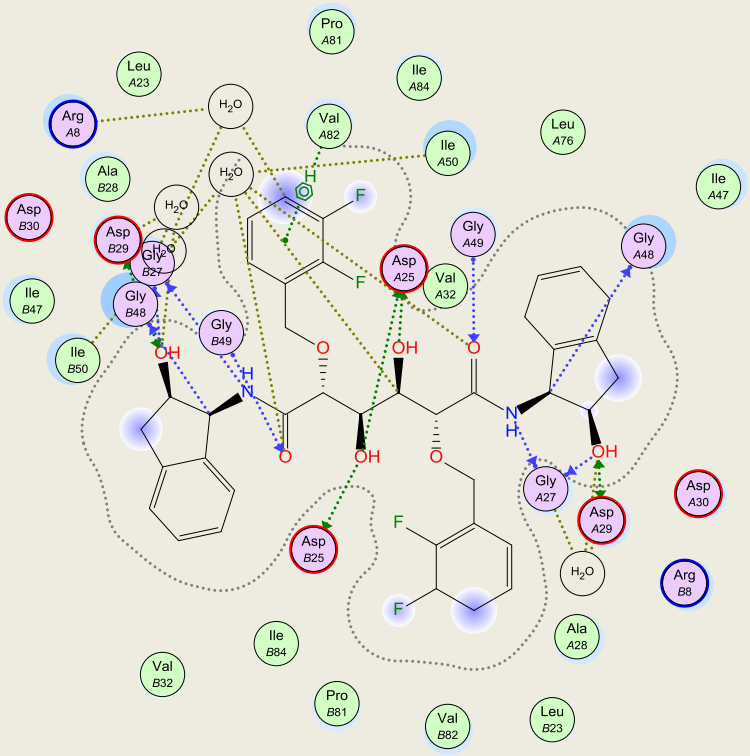
Practical Pharmaceutical Chemistry III